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THE PHARMACOLOGY OF THE AUTONOMIC SYSTEM

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A review of this subject, following so closely on the appearance of Langley's masterly little volume, *The Autonomic System*, requires unusual temerity. However, since most of the material had already been gathered and since the review had been planned from a somewhat different point of view, it seemed worth while to attempt to carry through the original enterprise; namely, a survey and orientation of the more recent developments that threatened or promised to disturb the narrow, rather rigid schema into which the pharmacology of the autonomic system had tended to "set."

The striking differences in the origin and distribution of the three great classes of efferent nerve-systems and their end organs—the somatic or voluntary system, the parasympathetic and the sympathetic—are reflected in an almost equally striking manner in their peripheral reactions to certain drugs; so much so that these could be definitely classed as sympathetic (for instance, epinephrin and ergotoxin); parasympathetic (for instance, pilocarpin and atropin); and strio-muscular (for instance, guanidin and curare). The correspondence between the drugs and innervations is so far-reaching that it cannot be accidental. There must be a fundamental, specific relation between the innervation and the drug-reactions; and if so, all poisons that act in a special manner on the peripheral system should belong, definitely and sharply, to one or the other of these groups; and we should thus be able to arrange all these drugs into a simple orderly, logical and generally satisfying system.

There is, however, another side of the picture: The correspondence is impressive, but not complete. On the one hand, some drugs affect, more or less, several of the systems; and on the other hand, the most

typical drugs seem to act on the wrong system in some organs, such as the uterus and the sweat glands. These exceptions were naturally set aside as "atypical" until the main scheme could be better digested; but they have continued to accumulate in number and in impressiveness until it seems that they might submerge the whole schema, unless the schema could be sufficiently broadened to cover them. Very evidently, the divisions of the efferent systems are not separated as sharply as the schema would imply; at least, the chasm between them is not impassable. The facts may be reconciled by the assumption that the fundamental distinction goes beyond the nervous systems; that the reactions, whether to drugs or to nervous impulses, depend on the physical or chemical properties of the reacting cell; that these vary with conditions; that a certain complexion of these conditions is ordinarily associated with each of the nervous systems; but that these conditions may be shifted, naturally and artificially; and that then the reactions to nerve impulses and to drugs are also changed, separately or together.

In other words, it would appear that autonomic specificity is not an absolute, separate entity; but that it is conditioned on the ordinary, fundamental properties of muscle, of reactive tissue; in fact, of excitability in general. Conditions which affect these are also capable, potentially, of affecting autonomic specificity. This specificity is relatively fixed, because these properties are relatively fixed in a given type of tissue.

Generalizations of this sort hold out attractive prospects of satisfying the innate longing for unification; but they have little value unless they unify actual phenomena. Perhaps the chief significance of the more recent work on autonomic drugs is therefore the collection of data concerning the conditions that modify response. As yet, these data can furnish only hints, partly because they are incomplete in themselves, and partly because their interpretation must await clearer conceptions of the various properties of excitable tissues than we possess at present. There is reason to hope that each will throw light on the other; and it would not be surprising if the paradoxical effects of autonomic drugs would be important aids in unravelling the complexities of the nature of excitation.

Incomplete as are the present data, the space at the disposal of this review does not suffice to present them with any degree of detail. It can only sketch the subject in its broadest outlines, with a bit filled in here and there, by way of illustration.

I. NATURALLY TRANSPOSED SPECIFICITY. Under this heading we shall consider the instances in which drugs of pronouncedly specific autonomic character act on the wrong system, *under normal conditions.* Drugs with more ubiquitous actions—nicotin, barium, histamin, pituitrin, etc.—will only be considered incidentally. They are inherently eclectic rather than specific; and although the distinction may in the end prove to be of degree rather than of kind, they would as yet tend to obscure more than to clear the subject of perverted specificity.

It would also be desirable to disregard quantitative anomalies for the present. The various autonomic organs require quite different doses of their specific drugs, so that some may appear to be relatively refractory. The difference may be merely a matter of penetration, or it may have a deeper significance. In any case, changes of concentration may lead to reversal of the effects on the same system; or bring out effects on the complementary system. The quantitative anomalies therefore furnish a bridge between atypical actions induced by artificial conditions (dosage), and those that occur naturally with the usual dosage.

A special difficulty arises in deciding whether a reversed response (for instance, the secondary acceleration of the mammalian heart by pilocarpin) is due to a reversed action on the same system (conversion of vagus excitation to vagus paralysis); or to a transfer of the action to the complementary system (vagus excitation to accelerator excitation). It might appear that this could be easily decided by resort to nerve-stimulation or to antagonistic drugs; but the results of these methods are often as difficult to interpret as the original problem.

It is suggestive that perverted drug specificity is confined largely to organs, in which the innervation is itself unusual or confused. It is especially common when the tissue receives only one innervation; or when one innervation predominates very largely over the other; or where both systems produce the same response. For instance, in the sweat glands, the genito-urinary system; the frog's lungs; the blood vessels; and in the unpublished instance of the frog's small intestine, perverted specificity of drug is generally associated with weak contrast in the specificity of innervation.

We may conveniently arrange the instances under the innervations that are affected:

1. Sympathetic Innervations affected by Parasympathetic Drugs. a. The sweat glands: These are often quoted as the most striking instance of perverse autonomic reaction. Those of the cat's paw were described

as innervated exclusively by the sympathetic, the electric stimulation of this causing secretion; but they fail to react to epinephrin, and on the contrary respond to the parasypmathetic poisons, to pilocarpin and atropin.

The work of Dieden and of Muto (1916), however throws doubt on the interpretation of the phenomena. They find that the sweat glands receive a parasympathetic as well as the sympathetic innervation; the relative importance varying in different animals. This would leave the possibility that the parasympathetic poisons act on a parasympathetic innervation. The drops of sweat that appear on stimulating the sympathetic could be due to contraction of the muscular coat of the glands, as suggested by Gaskell (p. 37); or the failure of epinephrin to produce secretion could be due to vasoconstriction.

The example of the sweat-glands is therefore not as decisive an instance of perverted specificity as is commonly supposed.

Incidentally, epinephrin has a normal augmentor effect on the sympathetically innervated skin-glands of the toad (Wastl, 1921).

b. The uterus: Although this has a double innervation, the parasympathetic appears to be practically negligible. Stimulation of the sympathetic (hypogastric) produces marked effects, which may be contractor or relaxor, according to the species of the animal. In cats, the response is inhibitory except during pregnancy, when it becomes contractor.

On pregnant cats, i.e., with contractor response of sympathetic stimulation, Cushny (1910) found that the parasympathetic pilocarpin produces contraction, as well as the sympathetic epinephrin. This has been abundantly confirmed, and seems to hold for all species and conditions in which hypogastric stimulation produces a contractor response.

The results as to the efficiency of the specific antagonists are conflicting. Cushny reported that the sympathetic ergotoxin antagonized the contractor effect of either drug; but that the parasympathetic atropin antagonized only the parasympathetic pilocarpin contraction, and not at all the response to epinephrin or to hypogastric stimulation. On the other hand, Dale and Laidlaw (1912) found ergotoxin ineffective against pilocarpin stimulation; and Gohara (1920) claims that atropin abolishes the contractor effect of epinephrin in rabbit's uterus.

There is also considerable uncertainty as to the transposition of inhibitory effects. In non-pregnant cats, in which hypogastric stimulation or epinephrin usually relax or inhibit the contractions, Cushny

also noted inhibitory phenomena after pilocarpin injection; but they were much less characteristic than with epinephrin. Dale and Laidlaw did not observe relaxation when the excised non-pregnant uterus was subjected to pilocarpin, although it occurred sometimes during life. This they attributed partly to increased suprarenal output, partly to action on the sympathetic ganglia, and not a peripheral autonomic affect. Gunn and Gunn (1914), however, did occasionally observe inhibitory effects on the excised uterus of rats and guinea pigs.

These conflicting data as to the exchange of antagonisms, and as to the acquisition of inhibitory sympathetic characters by pilocarpin, do not invalidate the high importance of the undoubted fact that the contractor sympathetic innervation responds identically to sympathetic and parasympathetic drugs. It is evidently not necessary that a drug should completely lose its identity when it changes from one system to the other; and one need not expect that the sympathetic affinity of pilocarpin should reach the high perfection of epinephrin.

c. Retractor penis: This has a typical double innervation, with definite reciprocal response; the sympathetic being constrictor, the parasympathetic inhibitory. There would thus seem to be no good reason for transposition; but Edmunds (1920) finds that pilocarpin as well as epinephrin are contractor; that the pilocarpin contraction is antagonized by atropin; and that atropin does not prevent the inhibitory response of stimulation of the parasympathetic nerve.

There is thus apparently a complete transposition of the parasympathetic drugs to the sympathetic system; one might say that the contractor affinity of pilocarpin is in this case more powerful than its parasympathetic affinity. However, the transposition is not quite complete; for the pilocarpin is not antagonized by the sympathetically depressant ergotoxin.

d. Urinary bladder: This has also a double innervation; the parasympathetic being contractor, the sympathetic rather complex. The drug responses apparently are essentially true to type; the anomalies that have been described being probably merely quantitative differences of susceptibility.

Edmunds and Roth (1920) find that the differences which Elliott (1907) had found between the response to epinephrin and sympathetic stimulation disappears with appropriate doses. They themselves report that the atropin does not paralyze the response of the parasympathetic electric stimulation, although it antagonizes pilocarpin and physostigmin. This, however, is found in many organs; the nerve current

seems more effective in overcoming the atropin block than are pilocarpin or physostigmin; or conversely, the dosage of atropin required to block stimulation of the parasympathetic nerves varies widely for different tissues (c.f. V. E. Henderson, 1922).

Other genito-urinary organs, so far as they have been studied, seem to have concordance of drug-specificity with innervation; whether this be double, as in the ureter; or predominantly sympathetic, as in the prostate (Macht, 1922).

e. Blood vessels: The evidence furnished by these is suggestive rather than crucial. They appear to have only a single innervation; at least, as judged by nerve stimulation, one nervous system or the other is so predominant that it appears exclusive. With most vessels this innervation is sympathetic and produces constriction. In the few situations where dilator effects have been definitely demonstrated, they are parasympathetic. However, drug reactions indicate that other vessels also have a mechanism for dilator stimulation through parasympathetic drugs, as shown most clearly for acetyl-cholin. This produces dilatation, which is antagonized by atropin (Hunt, 1918). Perhaps the parasympathetic "receptive substance" failed to establish connection with the nervous system because it is normally activated by the chemical products arising in the local metabolism.

The effects of pilocarpin are also dilator in most cases; but a few contractor reactions have been described, which would point to transposed stimulations of the sympathetic division. The data are cited by Langley (p. 37). Hildebrandt (1920) also reports that atropin antidotes epinephrin and sympathetic nerve stimulation in frog's vessels. It is not antagonistic to barium; but removes veratrin constriction (Kondo, 1919).

f. Heart: Pilocarpin inhibits the heart through vagus stimulation. This is followed by acceleration, expecially in mammals; in frogs, it removes muscarin standstill. This reversal is commonly attributed to direct depression of the vagus, which is found less responsive to stimulation. However, epinephrin also renders the heart irresponsive to the vagus (Langley, 1901; Kuroda and Kuno, 1916). This suggests that we may be dealing with a transposition of the pilocarpin stimulation to the sympathetic accelerators, and not with a direct transformation to parasympathetic paralysis.

g. Gastro-intestinal tract: One of the most striking instances of presumable transposition is furnished by the small intestine of the frog, whose behavior in saline baths has recently been studied at this labora-

tory by Dr. G. B. Roth (in press). It reacts in the usual manner to barium, by contraction; and to epinephrin by relaxation. The two drugs are mutually antagonistic, a matter of considerable interest for the localization of the barium action. Pilocarpin does not contract the intestine, as it does in mammals and turtles; or at most, there is a brief, faint and doubtful shortening; but to the contrary, it produces marked relaxation and antidotes the barium contraction, just like epinephrin. The pilocarpin relaxation is not antagonized by atropin, which is itself a depressant; although much weaker than pilocarpin. Physostigmin behaves like pilocarpin. The direction of the change does not appear to be influenced by the ion ratio or H-ion concentration of the bath.

In *other parts of the frog's intestine*, the pilocarpin and physostigmin response is in an intermediate stage: Fuehner (1918) found for the *stomach*, and Schuller (1921) for the *rectum*, that these drugs produce only a feeble contractor response; and in the rectum at least, pilocarpin prevents the contractor effect of the more fixedly parasympathetic arecolin.

A converse instance of the transposition of the parasympathetic poisons is furnished by the mammalian *ileo-colic sphincter*. Kuroda (1916) found that this is contracted not only by sympathetic stimulation and epinephrin, but also by parasympathetic stimulants; and that it is relaxed by atropin.

2. Parasympathetic Reactions provoked by Sympathetic Drugs. This transposition is less common; which conforms to the high degree of specificity of the sympathetic type of drug, epinephrin.

a. Lung-sacs of frogs and salamanders. The musculature corresponds to the bronchial muscle of mammals, but it has a different physiology and innervation (frog, Carlson and Luckhardt, 1920; salamanders, Luckhardt and Carlson, 1920). The muscle possesses a strong and continuous inherent tone, which is relaxed by parasympathetic (vagus) stimulation, and is slightly augmented by sympathetic stimulation. The response to epinephrin is transposed; i.e., it produces relaxation. Atropin presents another anomaly, for it does not prevent the inhibiting response to vagus stimulation. Pilocarpin which was tried only on salamanders, acts normally, i.e., relaxes.

In snakes and turtles, innervation and the response to drugs is as in mammals; contractor to parasympathetics; relaxor to epinephrin; except that the relaxor effect of epinephrin is still quite weak (Carlson and Luckhardt, 1920; Luckhardt and Carlson, 1921).

b. Blood vessels. As was discussed in a previous section, these generally respond by constriction to sympathetic stimulation; and by dilatation to parasympathetic stimulants. Although the sympathetic constrictor action of epinephrin is especially pronounced in the case of blood vessels, it produces dilatation in various situations; constantly so when lower concentrations of epinephrin are employed, or when the sympathetic excitability of the tissue has been lowered. It is not improbable that these are due to a parasympathetic transposition of epinephrin; this is strongly supported by the reaction of the pulmonary artery of frogs and turtles. In these animals, the constrictor innervation is not sympathetic, but parasympathetic (vagus; paralyzed by atropin); epinephrin, however, produces contraction, i.e., parasympathetic stimulation.

In other special vascular areas the drug-responses are still confusing. Considerable data for various drugs have been collected by the recent work of Amsler and Pick (1919) (frog-perfusion experiments, splanchnic areas and legs); Adler (1921) (ditto, lung and skin vessels); and Rothlin (1921) (rings of mammalian artery).

3. Autonomic Reactions provoked by Strio-Muscular Drugs. There seems to be a definite relation between the drug-affinities of the autonomic and strio-muscular innervations. Probably all of the drugs that act on the receptive mechanism of the striped muscles act also on the parasympathetic or sympathetic division, and most of the parasympathetic drugs act on striped muscle. The sympathetic division does not seem to affect muscular contraction and tone, but has been credited with influencing muscular metabolism.

The curare and nicotin group acts more especially on the autonomic ganglia, so that their effects on the terminal cells are not easily studied. *Guanidin* is perhaps more strictly terminal: The excised frog-heart (Rosenow, 1921) responds to weak concentrations by increased systole (sympathetic stimulation?); to higher concentrations by diastolic arrest (parasympathetic stimulation?). Bronchial muscle (Macht and Ting, 1922) is relaxed (sympathetic stimulation or parasympathetic depression?).

4. Strio-Muscular Reactions to Parasympathetic Drugs. The more powerful parasympathetic stimulants, physostigmin and acetyl cholin, stimulate the "receptive" mechanism of striped muscle in a manner strictly analogous to the characteristic strio-muscular stimulants, guanidin, nicotin and barium. The phenomena are the same for all, with mere quantitative differences that tend to disappear by varying the

dosage: *a*, The lowest effective concentrations produce fibrillary twitchings; with increasing doses these pass to clonic contractions, tonus contractures, and finally into curare-like depression. *b*, With all, the action is strongest at the site of the end-plate; which might mean that the receptive mechanism is concentrated at this place; or merely that the permeability of the cell-wall at this place is more favorable to the penetration of the drugs or ions. *c*, All continue effective for some time after the division of the nerve trunks. Most and probably all become less and less effective when the nerve has completely degenerated, presumably because the receptive mechanism, and eventually the muscle fibers as a whole, deteriorate when they are no longer used. *d*, All are antagonized, though to a varying degree, by curare, atropin and especially l-scopolamin, and the local anesthetic group. Atropin is more effective against drugs than against nerve-stimulation; a difference that it frequently exhibits in strictly parasympathetic reactions, and which is again a merely quantitative question, for sufficiently large doses of atropin block the response of striped muscle to nerve stimulation (Haffner, 1918).

The data for these conclusions, but not necessarily the interpretations themselves are chiefly taken from papers of Frank and Katz (1921) (local anesthetics, nicotin, guanidin); E. Frank and Nothmann (1921) (physostigmin and scopolamin, on human); Frank and Stern (1921) (guanidin, degeneration, procain, atropin, barium); Fuehner (1920) (guanidin); Haffner (1918) (atropin); Langley (1913 and 1914) (curare and nicotin); Magnus (1908) (nicotin, curare, physostigmin); Meighan (1919) (guanidin); Riesser and Neuschloss (1921 and 1922) (acetyl cholin, atropin, curare, nicotin, local anesthetics); Schuller (1921) (local anesthetics and caffein); Schuller and Athmer (1921) (local anesthetics and veratrin).

5. Sympathetic Innervation of Striped Muscle. There is practically nothing to add to the review of this subject given by Langley on pages 69–80 of his book. Briefly, sympathetic fibers have been traced to the striped muscle fibers; especially by the degeneration method (Dusser de Barenne and co-workers, 1919). It was suggested on speculative grounds that this sympathetic innervation controls the sarcoplasm, and through it the tone of the muscle, in accordance with Bottazzi's sarcoplastic theory of tone. De Boer (1916) claimed to have demonstrated experimentally a loss of tone on division of the sympathetic supply. Dusser de Barenne (1916)and S. Cobb (1918) showed that this has essentially no effect on the tone. The sympathetic innervation was

then credited with the regulation of the metabolism of the muscle, but without direct evidence. Langley points out the difficulty of distinguishing a sympathetic innervation of the muscles from a sympathetic innervation of the blood vessels in the muscles, and does not consider the evidence quite conclusive; but inclines to the positive side; i.e., that sympathetic nerves make direct connections with the muscle cells.

The behavior of striped muscle toward epinephrin supports the view that the sympathetic innervation does not act on muscular contraction or tone; for epinephrin does not affect these in excised muscle. On the other hand, epinephrin increases the carbon dioxid production, even in excised muscle (Martin and Armitstead, 1922), and also creatin formation (Riesser, 1916). This indicates that the sympathetic innervation may be concerned with possible functions of muscle in general metabolism; perhaps in heat-regulation.

II. Transposition of specific actions by artificial conditions. We have seen that normal specificity is not absolute; that the reactions of the parasympathetic and sympathetic drugs are sometimes transposed, sometimes intermingled. It is therefore evident that innervation, although the most important condition, is not the *only* condition concerned in the specificity of the autonomic drugs. These conditions could be reduced in principle to two, sensibility and dosage. Each of these in turn may be changed by a large variety of factors, of different relative importance.

Sensibility and dosage determine, in final analysis, the degree of normal action; reversion of normal action from stimulation to depression or conversely; and transposition to the other system. These are all reducible to the same principles. It will facilitate the exposition if we employ the following terms as indicated:

Reversal of function: Response to the organ in opposite directions (for instance, change of contraction to relaxation). This may be the result either of transposition or of transformation of action:

Transposition of action: From the normal to another system (for instance, an epinephrin effect on the parasympathetic innervation).

Transformation of action: The action being on the normal system, but in the opposite direction (for instance, a nicotin stimulation changing to a paralysis).

Sensitization and desensitization will be used for increased, respectively diminished, response of either normal or the transformed or the transposed actions.

Further discussion will be more profitable in connection with actual examples:

1. Action transposed by Intensity of Electric Stimulation: The claw of the crayfish is moved by a stronger adductor and a weaker abductor muscle. Either muscle can be placed in tone by the removal of the other. When the muscle, with its contained nerves, is then stimulated, the response varies according to the strength of the stimulus and the muscle:

Weak stimulation relaxes the adductor and contracts the abductor.

Strong stimulation contracts the adductor and relaxes the abductor.

Each muscle has a separate contractor and inhibitor innervation (the literature is cited by Gaskell, | p. 74–76). It is clear, therefore, that merely increasing the strength of the stimulus transposes it from the relaxor to the contractor innervation in the case of the adductor; and from the contractor to the relaxor innervation for the abductor.

The central nervous system furnishes numerous instances of transposition of action according to the strength of the afferent impulses, which may be more or less analogous; but it is advisable to confine ourselves for the present to peripheral reactions.

2. Paradoxic Cardiac Effects of Autonomic Poisons. In the excised frog heart, fed by the Straub cannula, the simultaneous action of sympathetic and parasympathetic stimulants does not result in neutralization of their effects, as might be expected; but in transposition of the stimulant action of one of the poisons to the other system. The Vienna school (Pick, 1920) therefore assumes that both groups of drugs may stimulate either of the autonomic systems, i.e., that they are really "amphotropic." They act, however, with different intensity; so that the stimulation of the complementary innervation becomes manifest only when its excitability is abnormally raised, or if the excitability of the usual innervation is abnormally depressed.

a. When under the moderate influence of parasympathetic stimulants (acetyl-cholin, etc.), the heart responds to epinephrin, not by the usual sympathetic augmentory response, but by increase of the parasympathetic diastolic, inhibitory action. This inhibitory effect is removed by atropin, so that it appears due to parasympathetic stimulation (Kolm and Pick, 1920). Analogous transposition is shown by the blood vessels and intestines.

Pick believes that the production by epinephrin of cardiac fibrillation and dilatation in early chloroform anesthesia is due to an analogous transposition, assuming that the vagus is sensitized by small doses of chloroform.

b. Nicotin and ergotamin also produce a similar transposition of epinephrin to inhibitory action (Amsler, 1920). Amsler assumes that the ergotamin paralysis of the sympathetic reciprocally increases the sensibility of the parasympathetic, and thus transposes the epinephrin action. This would also furnish the explanation of epinephrin "reversal" in other organs.

He also explains the nicotin-epinephrin phenomenon by paralysis of the sympathetic; but Hett (1920) finds that nicotin acts on the heart as a parasympathetic stimulant; so that the transposition may be more strictly parallel to the cholin-epinephrin phenomenon.

c. Physostigmin also sensitizes the heart to the diastolic action of strophanthin to such an extent, that even maximal doses produce diastolic arrest instead of the usual systolic standstill (Froehlich and Pick, 1920). Since systolic standstill seems to be connected with sympathetic stimulation, the strophanthin diastole may be conceived as a transposition.

d. Conversely, sensitization of the sympathetic by epinephrin, or by excess of calcium transposes the parasympathetic poisons (muscarin, acetylcholin, etc.) to sympathetic stimulation; so that they produce systolic contracture instead of diastolic arrest. This sympathetic action of physostigmin is prevented by ergotoxin, and not by atropin.

e. Abolition of ventricular vagus response by excessive doses of vagostimulants. The mechanism of this phenomenon is not clear; but it may receive mention in this place. Large doses of apparently all vagus stimulants (aconite, muscarin, physostigmin, digitalis, acetylcholin, pituitary) render the ventricles irresponsive to vagus stimulation. The sinus and auricles still react by diastolic standstill, so that the interference appears to be with the conduction of the inhibitory impulses (Froehlich and Pick). It is therefore possible that the action does not consist in reversal to the vagus effect, but that it may involve some other mechanism of the heart.

3. The Influence of Electrolytes on Cardiac Response. The ions of serum and saline solutions influence profoundly all the functions of the heart, and among them their response to autonomic stimulation by drugs. These ion effects are very complicated, confusing and difficult to analyze; partly because they depend on the ratio, for instance of Ca:K, as well as on the absolute quantity of each; and partly because different levels of the heart react quite differently, and sometimes in opposite directiosn. The practical importance of this latter fact is not yet sufficiently widely realized; for it determines the gradient of irritability, of

tone and of rhythm production; so that the "pace-maker" may be shifted at will by changes in the ion ratio (Sakai, 1914; Daly and Clark, 1921; Kolm and Pick, 1920). Presumably this applies also to peristaltic progression in general; for instance in the intestines. However, changes in ion-ratio sufficient to influence autonomic reactions are not readily produced in intact animals (Schafer, 1915).

A detailed discussion to the ion-effects would be beyond the scope of this review. The more recent data can be found in the papers of Daly and Clark, Kolm and Pick and Carter and Andrus (1921).

a. In general, changes in the potassium and calcium content act in opposite directions:

Increase of the $\frac{K}{Ca}$ *ratio* (i.e., *Calcium deficiency or potassium excess*)

produces the typical phenomena of parasympathetic (vagus) stimulation, culminating in diastolic standstill (e.g., Burridge, 1912).

Increase of the $\frac{Ca}{K}$ *ratio* (i.e., *Calcium excess or potassium deficiency*)

produces the phenomena of sympathetic (accelerator) stimulation, culminating in systolic standstill (Kolm and Pick, 1920).

In brief then, *calcium* seems to sensitize the sympathetic or desensitize the parasympathetic, and *potassium* seems to sensitize the parasympathetic or to desensitize the sympathetic mechanism.

(These simplified statements hold only for solutions that contain Ca, K and Na ions. The phenomena are modified if one of the constituents is entirely absent; for instance, entire absence of Ca abolishes the response to vagus stimulation (O. Loewi, 1917).)

b. When the ion-changes are combined with the other autonomic stimulations or drugs, they reinforce or transpose the effects, in the same direction as if the ordinary specific autonomic poisons were combined:

Increase of the $\frac{\mathrm{K}}{\mathrm{Ca}}$ *ratio* (*Calcium deficiency or potassium excess*),

corresponds to sensitization of the parasympathetic (vagus) and enhances the response of the parasympathetic stimulants (muscarin, cholin, etc.) and transposes the action of sympathetic stimulants, so that the epinephrin now produces a diastolic effect (Burridge, 1912), which is antagonized by atropin, but not by ergotoxin (Kolm and Pick, 1920).

Increase of the $\frac{Ca}{K}$ *ratio* (*Calcium excess or potassium deficiency*) corresponds to sensitization of the sympathetic (accelerator) and therefore enhances the response to epinephrin, and transposes the action of parasympathetic stimulants, so that acetyl-cholin produces systolic instead of diastolic standstill. The action is antagonized by ergotoxin, not by atropin (Kolm and Pick).

Increase of the $\frac{Ca}{K}$ ratio also sensitizes to systolic standstilll by *strophanthin*, whilst increase of $\frac{K}{Ca}$ causes strophanthin to produce diastolic standstill (Loewi, 1915).

c. Sensitization of the sympathetic or desensitization of the parasympathetic is also produced by non-electrolytes; by lecithin and soaps; by serum; by increase of alkalinity; and by seasonal changes:

The presence of *non-electrolytes* (urea, sugar) renders the heart more resistant to calcium deficiency; which Loewi (1921) interprets as sensitization to the smaller quantities of calcium that remain. The non-electrolytes also sensitize to strophanthin; they may therefore be classed with the sensitizers of sympathetic action.

Treatment of the heart with *lecithin* and *soap* renders it more resistant to cholin and pilocarpin, i.e., diminishes parasympathetic stimulation and would therefore sensitize to sympathetic stimulation (Loewi, 1921).

Blood serum antagonizes parasympathetic stimulation (muscarin), and increases the parasympathetic depression of atropin. This is due partly to its calcium, partly to the ether-soluble extractives (Kirste, 1921).

Increase of *alkalinity*, within physiological limits (to H_p–7.8) increases the tonus (Andrus, 1919); i.e., acts in the sympathetic direction.

The Aestival vagus insusceptibility of frogs is not due to changes in the nerves, but to desensitization; for the inhibitory response can be restored by drugs that stimulate the vagus or that depress the sympathetic (Cori, 1921).

Radioactive substances: These seem to act in the direction of parasympathetic sensitization; for they seem to have the effect of radio-equivalent quantities of potassium (Zwaardemaker, 1918). Zwaardemaker suggests that the radio-energy liberates potassium (and presumably other ions) from their combinations in the protoplasm.

4. Paradoxic Vascular Reactions, and Influence of Electrolytes: The blood vessels exhibit the same transposition of specific autonomic drugs by sensitization of the opposing system or desensitization of the normal system, as was described under the heart; either through specific autonomic drugs, or through changes of the ions in the salt mixture, or through other means.

a. Dilator action of epinephrin. The ordinary constrictor action of epinephrin is identified with sympathetic stimulation. Under certain conditions this is replaced by a dilator reaction, which is presumably dependent on parasympathetic affinities; this becomes manifest especially whenever the sensitivity of the sympathetic innervation is lowered; as by degenerative nerve-section (Pearce, 1913; Engelock, 1915); by prolonged sojourn of the tissue outside of the body (Sollmann, 1905); or by very prolonged perfusion with epinephrin itself (Ogawa, 1912; Bauer and Froehlich, 1918); and by autonomic drugs and ions that depress sympathetic or increase parasympathetic reactivity.

Very small doses of epinephrin are also credited with a dilator action; it is not known whether this depends on the same mechanism.

The normal dilator response of certain vessels (studied by Rothlin, 1921) may be due to the normal preponderance of parasympathetic irritability.

b. Sensitization by specific autonomic drugs. The transpositions through the presence of other specific autonomic poisons are identical with those described in section II, no. 2, for the heart (Kolm and Pick, 1920).

Spleen extract also sensitizes to the pressor (sympathetic) response of epinephrin; and antagonizes the depressor (parasympathetic?) response (Collip, 1920).

c. Relation to electrolytes. Calcium appears to act in the opposite direction in vessels, and in the heart. Potassium and hydrogen and hydroxyl ions act on the whole in the same direction in both; (Schmidt, 1921, frog perfusion):

Calcium excess acts as a parasympathetic stimulant to the vessels, and as a sympathetic stimulant in the heart. The vessels are dilated, and if the constrictor response to sympathetic nerve stimulation and to epinephrin is diminished, they may even respond by dilatation.

Calcium deficiency acts as a sympathetic stimulant on the vessels, and as a parasympathetic stimulant on the heart. The vessels are constricted, and the constrictor response to epinephrin is enhanced.

Potassium excess acts as sympathetic stimulant to the vessels; as a parasympathetic stiumlant on the heart. The vessels are constricted. However, the effect on epinephrin is abnormal; the constrictor effect is diminished, or may even be replaced by dilatation.

Potassium deficiency also acts as a sympathetic stimulant, to the vessels, as well as to the heart. The vessels are constricted and react more effectively to the constrictor action of epinephrin.

Alkalinity increase: Moderate increase acts in the direction of sympathetic stimulation for the vessels, as for the heart; i.e., perfused vessels constrict. This is not antagonized by ergotoxin. Epinephrin is effective (Heymann, 1921). Moderate alkalinity also promotes the contractions and tone of excised arteries (Opitz, 1920).

Large excess of alkalinity relaxes (Heymann).

Diminished alkalinity and increased acidity: Diminished alkalinity also constricts the perfused vessels (Pearce, 1913). The mechanism is not clear; the constriction is prevented by ergotoxin, so that it might be considered as sympathetic; but the constrictor response to epinephrin and to nerve stimulation is abolished or replaced by a dilator response (Heymann; Schmidt).

A larger excess of acid dilates the vessels; the dilator concentration coinciding with the H ion concentration that stimulates the respiratory center (Adler, 1921).

Transposition of action by sensitization and desensitization applies also to the other organs on which it has been studied; for instance, the intestines (Kolm and Pick). It is not necessary to occupy space in their discussion further than to point out that many details remain to be elaborated.

III. Peripheral point of attack of the autonomic drugs. The energy and ingenuity that have been spent on attempts to localize the site of the action of autonomic drugs has not yet borne full fruition. Indeed, the subject has been full of disappointments—or of interest, according to the point of view. Again and again, conclusions that appeared securely established had to be revised. Methods of experimentation and deduction that appeared conclusive were found, on further examination, to be inadequate and misleading. Differences that appeared fundamental turned out to be only quantitative, subject to exceptions, dependent on conditions. Magnus in 1908 destroyed confidence in localization by antagonists, but considered anatomic methods of isolation as conclusive; it has since been shown that these may also lead to false conclusions, by being incomplete, or by injury to

other tissues, or by indirect reactions through tone, reciprocal innervation, etc. Even degeneration of nerve-endings has led to deceptive conclusions.

This experience may seem discouraging as to theories; but it is inspiring to the search for facts: for the destruction of the theories has been brought about by, and led to, the accumulation of data. The theories have therefore served their normal useful fertilizing function.

To proceed with the consideration of the points of attack: Autonomic drugs could conceivably stimulate or depress either of the two systems by acting on four principal mechanisms:

1, On the ganglionic elements; *2*, on the nerve-endings; *3*, on the receptive mechanism or excitability of the terminal cell; and *4*, on the responsive mechanism, the contractility or secretory activity.

1. The Ganglionic Elements. The ganglia of invertebrates are susceptible to the whole series of autonomic drugs; for instance, the ganglion-chains of the marine worm, sipunculus (Magnus, 1903); or the cardiac ganglia of the king-crab (Carlson, 1906). With vertebrates, the autonomic ganglia react definitely to the nicotin group, as shown by the fundamental work of Langley; but wherever they can be separated with sufficient sharpness, they do not seem to be very susceptible to the other sympathetic and parasympathetic poisons. It is more difficult to confirm this conclusion absolutely in situations where they are inseparably intermingled with the terminal elements, as in the heart and in the intestines. The analogy of the limulus heart lent color to the suggestion that the ganglion cells might be the seat of the rhythmic activity and of the actions of drugs upon it. The analogy, however, is not binding, and the "neurogenic theory" has almost hopelessly lost ground for the vertebrate heart (Eyster and Meek, 1921). As concerns the autonomic drugs these act on the ganglion-free apex essentially as on the whole heart (S. Loewe, 1918; Sasaki, 1921).

The evidence for the importance of the intestinal ganglion plexus to the autonomic reactions rested essentially on the experiments of Magnus (1904) that seemed to show that the rhythmic contractions of the intestines, and the effect of drugs upon them, depended on the integrity of the Auerbach plexus. Later work, however, showed that the absence of rhythm was due merely to traumatic injury; Gunn and Underhill (1922), showed that plexus-free preparations are rhythmic; and that the rhythm can be evoked for days after the excision, whilst the ganglion cells are assumed to die quite promptly. This has been abundantly confirmed (for instance, Alvarez and Mahoney, 1922); and holds true

also for the peristaltic movements of the excised intestine, as shown by the method of Trendelenburg (1917). The results of these indicate that origin and direction of the peristaltic wave depend on a gradient of tonus and excitability, inherent in the muscle; which in turn depends presumably on adjustment to the optimum ion ration. The effects of drugs in revived intestine also agree essentially with those in intestine in which the ganglia are alive.

Carlson and his co-workers find another argument for the independent activity of the Auerbach ganglia in the capricious response of the cardial region of the stomach to nerve stimulation: either vagus or sympathetic may produce either contraction or inhibition (Carlson, Boyd and Pearcy, 1922). From this they argue that these nerves do not act directly on the muscle, but that they are largely afferent nerves to peripheral reflex center, i.e., to the ganglion plexus. The deduction does not appear conclusive. It is conceivable that each nerve might carry both augmentor and inhibitory fibers directly to the muscles (as Tashiro (1920) assumes for the intestinal sympathetic), or end result of the same stimulation on a given muscle fiber may be modified by the state, especially the tone, of that fiber. On the whole the facts seem in harmony with Gaskell's view that the ganglion cells of the autonomic system are connector elements, analogous to the pyramidal cells of the motor system, but transplanted to the periphery. However, the final conclusion must be left to the future.

2. Nerve-Fibrils and Endings. Nerve-fibers, where they can be reached separately, are practically unaffected by most of the specific autonomic poisons. It is therefore improbable that the finer terminal fibrils, which seem to be mere continuations of the axis-cylinders, should be the site of the specific actions. They differ from the larger fibers in permeability, it is true; but this would cause only quantitative differences, as is shown by the local anesthetics.

Direct evidence against the nerve fibrils is furnished by the persistence of the responses to autonomic drugs in excised organs after the excitability of the nerve fibers has been lost.

The results after degeneration of the nerve fibrils and endings, following division of the nerve trunk, speak in the same direction. The effects of a number of autonomic drugs, notably epinephrin, can still be evoked after complete degeneration of the nerve. This localizes their action definitely beyond the termini that are in nutritive connection with the nerve.

The interpretation of the converse phenomenon, disappearance of the drug response as the result of degeneration, is not so decisive. The effects of pilocarpin, for instance, can be evoked like those of epinephrin, after degeneration, i.e., when stimulation of the nerve trunk is ineffective. After a time however (6 weeks for sweat, Anderson and Langley, 1904) the response becomes less efficient although the muscle still responds to electric stimulation. This probably means that in the end the muscle cell also deteriorates, "atrophies," when it is not functionally used; and that this deterioration abolishes its excitability to pilocarpin before it abolishes the contractor response to direct electric stimulation.

The response to physostigmin disappears in general earlier than the response to pilocarpin: For the eye, within a few days (Anderson, 1905); but for the fibrillary contraction of striped muscle only between the 18th and 27th day (Magnus, 1908). The graduation suggests that the same mechanism is involved as with pilocarpin, i.e., gradual degeneration of the muscle.

It is rather unexpected that the excitability to physostigmin should disappear before the excitability to pilocarpin, which is a less powerful stimulant for innervated muscle, and the less powerful antagonist of atropin; but that does not prove that physostigmin acts on the nerve endings. It may be, for instance, that the physostigmin requires a special configuration of the receptive mechanism that degenerates relatively more rapidly; or it may be that pilocarpin actually excites the receptive mechanism, whilst physostigmin acts more as a sensitizer to other excitations, which would be excluded as degeneration proceeds. In favor of this explanation is the fact that the smaller doses of physostigmin only lower the threshold of muscle (normal) to nerve stimulation; that somewhat larger doses cause fibrillary twitching only after electric stimulation, and that spontaneous twitchings require a still higher dosage (Langley and Kato, 1915).

It may therefore be concluded that there is no conclusive evidence that any of the specific autonomic poisons act on the "nerve-endings" that degenerate after section of the nerves; and that the evidence of excised organs is distinctly against this mechanism.

3. The Receptive Mechanism. The fact that epinephrin contracts some smooth muscles, and relaxes others of apparently identical structure and which respond alike to direct electric stimulation, implies that it does not act directly on the contractile muscle substance. The striking correspondence with the response to sympathetic nerve stimulation implies that it acts on a nervous mechanism, but the persistence of the

response after nerve-degeneration proves that it does not act upon the nerves proper. It therefore became necessary to assume the existence of some intermediate mechanism, related to the innervation, but not trophically dependent upon it, on which the epinephrin and perhaps the nerve current must act. Epinephrin does not stand alone in this respect; probably all specific autonomic poisons act through the mediation of a recipient mechanism; and even some poisons that were formerly believed to affect the contractile substance directly.

The intermediate mechanism may be conceived in several ways: *a*, as a morphologic structure; *b*, as a chemical receptive substance or side-chain; *c*, as a physical system. The available facts do not permit a decision between these; indeed, the theories themselves lack so much in detail that a decisive attitude toward them would not be justified. Consequently, there is an advantage in employing a non-committal term, such as "*receptive mechanism*" for the entire concept; and to restrict the more specific terms, "myo-neural junction," "receptive substance," and "synapse," to the special theories that they represent.

These theories should be examined in the light of the characteristic phenomena of the autonomic system: the fact that the autonomic cells are usually innervated by two systems of nerves; that either of these may be augmentory or inhibitory in different situations; that autonomic drugs act specifically on one or the other of these systems, in one direction or the other; and that these actions may be transposed or transformed. Any theory of the receptive mechanism must be compatible with these phenomena, and if possible seek to explain them. This task may not prove as formidable as it sounds.

a. Morphologic structure intermediate between nerve and muscle; the "myo-neural junction" of Brodie. This assumes that the nerves are continued into the muscle cell, and there become modified in such a way that they do not degenerate on section of the nerve trunk. This attempts only to account for the persistence of the autonomic drug-response after nerve-degeneration; it leaves untouched all the other phenomena we have indicated and is difficult to reconcile with some of these, for instance, the transposition of action. The direct evidence for it is slender and dubious; and even if non-degenerating endings exist, this need not mean that they are the site of the action of the drugs; and if they are, this could be made a part of either of the other theories.

The direct evidence for the intermediate structure consists essentially of the "endplates" of striped muscle: these are definite structures, which might possess modified nervous functions; and the nerve fibrils

themselves apparently lie within the sarcolemma, in close apposition to the nuclei, which might modify their nutrition. However, Langley (p. 49) cites Boeke that the intracellular network does degenerate after nerve section, which speaks against rather than for the myo-neural theory; and the nucleo-sarcoplastic part of the sole-plate cannot be invoked, since nothing of the sort is present in the cells of the autonomic system proper.

b. Chemical receptive substance. Langley assumes that the autonomic drugs combine chemically with constituents of the cell—the receptive substances, or rather perhaps labile side-chain receptors of the molecule of the general cell substance. He considers that two classes of these receptors are necessary, one giving rise to contraction, the other to inhibition; the response to sympathetic or parasympathetic stimulation depending on the relative amount of contractor and inhibitor receptive substance "connected with them in the cells."

The two receptive substances could be made to account for the response to nerve impulses, but it seems difficult to apply them to their original object, the response to drugs. It is not clear, for instance, on what epinephrin is supposed to act. If simply on the receptor substance, then why should it have any relation to the sympathetic nerve supply? If it acts on the receptive substance only if this is connected with the sympathetic nerve supply, then the receptor theory loses its chemical character and becomes essentially a theory of specific difference in the nerve itself—a supposition that Langley himself rejects. The only alternative seems to be to assume four receptive substances—sympathetic contractor, sympathetic inhibitor, parasympathetic inhibitor, and parasympathetic contractor; and by buttressing this with a sufficient number of other subsidiary assumptions, explanations could probably be devised for all the autonomic phenomena; but one could not have much confidence in so elaborate a superstructure built on such slender foundation. It is safer to return to the general conclusion that the action of autonomic poisons *may* be chemical, and *may* be exerted on the more labile portion of the general molecule of the cells; there is really no evidence that even this is really the case.

c. The physical system of the cell as its receptive mechanism. The Nernst theory of stimulation, i.e., changes in the electrical potential of the cell, can be made the basis of interesting explanations for the receptive mechanism and its phenomena:

The double innervation with usually contrary response could be explained by the termination of the nerves at regions of different poten-

tial in the cell, which we shall call A and B. The contrasting regions could be nuclear and contra-nuclear; central and polar; membrane and sarcoplasm; exterior and interior—it is not necessary to settle this for the present. For the sake of illustration, we shall assume the nuclear and contranuclear regions, as these are undoubtedly at different potential. As the nerves develop in the embryo and grow out to make connections with the muscle or gland cells, they would naturally be directed to one region or the other of the cell, by this polar distribution of energy, or by other causes. It may be imagined that this direction would differ for the two systems of nerves, if these present different chemical or energy conditions—due perhaps to their different distance from their nutritive ganglion cell. The parasympathetic innervation could thus be attracted to region A and the sympathetic to region B—the nuclear and contranuclear region; the exterior and the interior of the cells, etc. The relative importance of these two regions may be conceived to vary for different types of tissue, and with conditions, so that either the sympathetic or parasympathetic innervation may be dominant. It is also possible to imagine that in the cell of some regions conditions exist that reverse the directive forces, and thus account for reversed innervations; or that the directive force is so nearly neutral that both innervations connect more or less with one region, or with both regions.

The physical schema also offers a plausible picture of the direction of the response: Let us assume that approach to a certain electric equilibrium, say to the iso-electric point of the protoplasm, causes contraction of the cell, through surface tension, imbibition, osmosis or some such process; and that departure from this point causes relaxation. Increase of potential at region A would affect the electrical equilibrium of the cell in the opposite direction from increase of potential at region B of the same cell; and thus contraction or relaxation would ensue according to whether the nerve connected with region A or with region B. This would explain the usual reciprocal character of the two innervations.

It does not follow that stimulation of region A produces the same direction of response for all cells; it need not even be always uniform for any one cell. The response would be influenced by the electrical condition of the cell; just as the iso-electric point of a protein is either approached or receded by the addition of an acid, according as the original protein was alkaline or acid. This may be the key to the phenomena of inhibition by the dominant innervation; to paradoxic

responses to nerve stimulation; to the effects of ion-ratios; to various colloid and anaphylactoid phenomena; and to the specificity of autonomic drugs.

These drugs may be conceived as acting chemically or physically on the cell in a large variety of ways; by producing precipitation or solution; changes of the ion-permeability of the cell membranes or of the protoplasm itself; changes in surface tension; changes in the size and surface charges of the colloids; changes in oxidation, reduction, cleavage or synthetic processes; direct chemical combination with the cell constituents, etc. Whatever the change, it must necessarily lead to a change of the distribution of potential within the system. The change produced by any particular drug must tend to react more upon one region than upon the other; and this results in more or less specificity. The high specificity of pilocarpin and epinephrin suggest that these act very predominantly at opposite regions, to produce the same changes of potential as nerve stimulation; but the phenomena of transposition suggest that they act somewhat throughout the cell, the dominant response depending on conditions, i.e., on "sensitization." Such conditions could be created by the simultaneous presence of both drugs; or by disturbing the energy distribution of the system by changing the ion-permeability of the cell wall by calcium or potassium, etc; or in anaphylactic sensitization perhaps by a change in the size of the colloid aggregates.

4. The Responsive Mechanism: The sharp separation of this from the receptive mechanism is of doubtful validity: the functions of excitability and contractility or secretion are so closely related that it is impossible to imagine a muscle that is excitable but does not contract; or one that contracts but it not excitable. The separation can only be made in a restricted sense; i.e., a muscle may be inexcitable for some particular kind of stimulation, but respond to others. In practice, the idea of receptive mechanism has been restricted to the transfer of nervous excitation and the excitability to specifically autonomic drugs; i.e., stimulations that may be conceived to act more especially on the contrasting A and B regions. Drugs like barium, caffein, the nitrites, the isoquinolin group, etc., seem to have less specific relation to these regions. They could be conceived as acting more generally throughout the cell; but it is again unprofitable to go far into details at present. It is difficult to imagine how they could leave the energy-distribution quite unchanged. Indirectly, at least, they must affect the irritability to all forms of stimuli.

The general physiological conditions of the cell must similarly influence its specific irritability, at least quantitatively. This is illustrated by the influence of tone and rhythmic activity on the response to autonomic poisons.

Space does not permit the further development of this subject, nor of many other incidental problems that are highly interesting, but which have less direct application to the basic phenomena that characterize the phenomena of autonomic drug response. It is a temptation to cite these at least in the bibliography; but even this would extend the review far beyond its allotted space. The following titles are therefore, confined to the material that is directly quoted in the review.

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THE LEUKOCYTES

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To Hewson we owe the discovery of the leukocytes, the early recognition of their importance in pathological processes we owe to Virchow, and to Ehrlich we are indebted for the development of a technique which made possible their differentiation. Ehrlich's classification of leukocytes, based on morphology and specific tinctorial reactions of protoplasmic granules, forms the base-line for almost a half-century of investigation of the varied hematological problems thus opened up. There has grown up in this period a literature of such tremendous volume that its review approaches the impossible and would in fact be undesirable, as, in the course of time, there has developed a certain stabilization of knowledge of the subject which renders many earlier discussions and opinions obsolete. My purpose therefore in this paper is rather to summarize or restate present-day opinions concerning leukocyte biology.

Any discussion of leukocytes must begin with a classification of the forms found in the circulating blood of the adult animal and the classification of Ehrlich is the one generally accepted, with, however, various reservations as to interpretation of forms and their relations. One must recognize three major groups of cells: the granular group of Ehrlich (the granulocytes), the lymphocytes, and the large mononuclear transitional group (the monocytes of Naegeli). The last two groups form the non-granular cells of the Ehrlich classification. That this grouping is valid and represents in all probability a fundamental variation in function of the different types of cells is clearly indicated by the fact that similar types of cells are found, not only throughout the vertebrate series of animals, but also quite generally throughout the invertebrates (Kollmann).

However, the claim of the Ehrlich school that the particular chemical reaction of the characteristic granules of the granular group of cells is necessarily a criterion of specific function has been challenged and possibly justly. Weidenreich in opposition to the Ehrlich view emphasizes the well-known fact that, while in man the granulation of the dominant polymorphonuclear cell has a neutrophilic reaction,

in the rabbit the morphologically and functionally equivalent cell has an eosinophilic (pseudo-eosinophilic) reaction, in the guinea pig amphophilic, and in the rat and mouse granules are undemonstrable by use of the ordinary strains. It is further recognized that within a single species of animal the reaction of one type of granule may change in the course of its development. For example, in the guinea pig the granules in the developing eosinophilic myelocyte are basophilic in reaction (Downey). Even in the circulating blood in man, in a case of sharp eosinophilia (60 per cent of 13,000 leukocytes), I have noted basophilic granules among the eosinophilic and even cells in which granules of obvious eosinophilic size and character were entirely basophilic in reaction. Yet it must be admitted that the granules of the mature eosinophilic cells all show definitely an eosinophilic reaction. In spite of these variations in reaction, one must admit that in the circulating blood there are three distinct types of mature granular cell, the neutrophilic, eosinophilic and basophilic and that the distinct granules must in all probability be related in some way to the different functional activity of the cells. I am not willing to accept Weidenreich's conclusion that the neutrophilic is the only granule in the leukocytes of man which is of endogenous origin and the result of protoplasmic activity.

The application of the Romanowsky type of stain to the leukocytes early revealed an error in the Ehrlich designation of the lymphocyte and large mononuclear groups of cells as non-granular, hyalin types. The lymphocyte is shown to contain a few coarse,—at times rod-shaped —granules, with a metachromatic reaction, while the large mononuclear group shows a fine azurophile granulation. That these granules may be as important functionally as those of the so-called granular group of cells is not to be doubted.

This gives us then five distinctive types of granules in five separate and distinctive types of cells in the normal circulation of man; the polymorphonuclear cell with fine neutrophile granules; the polymorphonuclear (usually bilobed) cell with coarse eosinophile granules; the cell with irregular nucleus (often polymorphic, sometimes round) with coarse basophilic granules; the small lymphocyte with a few metachromatic granules; and the large mononuclear (or transitional of Ehrlich) with a fine azurophile granulation. There is no tendency in medical literature at the present time to regard these as other than independent forms,—end-cells—with no development from one type into another. The Ehrlich term "transitional" for the large mononu-

clear cell with indented or lobed nucleus, has long been recognized as a misnomer. The cell does not become a polymorphonuclear neutrophile, as was supposed. There has been a shifting of discussion from the question of possible relationship and transition between these mature cells to the question of relationship between their ancestor-forms, and, further, of the relationship of these stem cells to the ancestor of the red blood cell series and to endothelium. As it is universally agreed that the primitive parent of all blood cells is an undifferentiated embryonic mesenchyme cell, the moot point is the question as to how early or how late the totipotentiality (as far as the blood is concerned) of this cell is lost. Does differentiation take place early in embryonic life, or does there survive in lymphoid tissue (including the red marrow) until late in adult life or in fact throughout adult life, a cell that is still totipotential? This question is the Shibboleth which has divided the camp of the hematologists. One group, following the lead of Ehrlich, accepts the answer of an early embryonic differentiation of cells, and constitutes the so-called polyphyletic school. While this school recognizes the presence of immature, undifferentiated cells in the hematopoietic tissues of the adult, yet they would assign to such cells the power to develop along but a single line predestined from the embryonic period. These cells, then, morphologically indistinguishable from each other, are potentially wide apart. The monophyletic school, on the contrary, following the fundamental studies of Pappenheim, Maximow and others, recognizes in an indifferent free amoeboid mesenchyme cell of lymphocyte character, with basophilic granule-free protoplasm, the parent stem cell of all types of blood cells—a polyvalent cell, persisting in lymphoid tissues throughout life. The line of development of this cell depends upon the type of external stimulus and not upon inherent latent tendencies.

The embryology of a fixed tissue is difficult, but how much more difficult the determination of relationship in a group of cells which are not only capable of independent motion and early wander from their place of origin, but which also are soon caught in the whirl of a circulating medium. In addition to general recognition of origin from a mesenchyme cell, there seems to be agreement that in all animals the first blood formation takes place in the yolk sac. Beyond this point one is forced to conclude that there must be variation in different orders of vertebrates or that observation is of such difficulty that personal opinion weighs heavy in the conclusion, for even in the later embryological studies there is wide divergence of opinion.

Thus Stockard in a study of the living yolk sac of a Teleost, (Fundulus heteroclitus) finds that four distinct types of cells, endothelium, hematoblasts and two types of chromatophores, develop in the same environment from apparently similar wandering mesenchyme cells. His conclusion is that these cells must have been differentiated before they wandered from the point of their origin else the identical external conditions should have produced differentiation along but a single line. In further experiments, in which the heart's action and the development of a circulation was prevented by alcoholic intoxication, he finds that although vessels are formed, they contain no red blood cells and there is not the slightest evidence of red blood cell formation from endothelial cells. A further early differentiation or predestination he believes is indicated by the fact that the red blood cells arise in the posterior part of the embryo, while the leukocytes arise later and from the anterior region.

In birds, on the other hand, Sabin, working with living yolk-sac membranes, finds that there is first developed from the mesenchyme an angioblast, from which are derived not only endothelial cells but also red blood cells. The latter may also arise from more mature endothelial cells. The endothelial cells, further, proliferate, giving rise to large mononuclear cells of the blood and the clasmatocytes of the tissues. The forerunner of the granular leukocyte of the chick appears in tissues in close proximity to blood vessels and is at first not to be distinguished from a single angioblast. It is only surely distinguishable by its behavior after division. Two angioblasts cohere, leukoblasts separate. Sabin does not recognize a primitive common blood stem cell or "hematoblast" and apparently does not wish to identify her "angioblast" as such.

Danchakoff, however, studying embryonic chick material, concludes definitely that there does separate from the primitive mesenchyme a parent blood cell—the "hematoblast"—which develops into red blood cells if caught within a developing blood vessel, and into a leukocyte if it lies in a different environment, that of the extravascular tissues.

Thiel and Downey studying hematopoiesis in the spleen of the embryo pig, find that a "hematoblast" frees itself from the mesenchyme and develops into red blood cells chiefly in extravascular spaces. While few leukocytes are formed at any time in the spleen, they apparently arise from a similar or the same parent stem cell. Lymphocytes separate from mesenchyme cells directly without an intervening hematoblast stage.

It is difficult to reconcile such varied findings as these with the assumption of a similar type of development of blood cells in all types of vertebrates. One might perhaps better conclude it is another case of scales, feathers and hair. Gross variations in hematopoiesis and in mature cells are found in adult animal forms of the different groups. It is well known, for example, that in the adult bird, red-cell formation takes place intravascularly, while in the mammalian marrow it occurs definitely extravascularly. The loss of nucleus from the mammalian red corpuscle is another well-known deviation from the blood of lower vertebrate forms. The variation in type of the special granules of the polymorphonuclear leukocyte, seen among the members of the mammalian group, has already been referred to.

In spite of the lack of desired proof from embryological studies, there appears to have been a growing tendency to accept the unitarian or monophyletic doctrine for the origin of the blood cells, even in adult life. The polyphyletic doctrine, allowing in the adult only homoplastic origin of blood cells, like cell from like, seems too narrow to explain many pathological phenomena of hematopoiesis. It postulates too sharp a specificity of cells. The problem is practically the same as that with which one is confronted in the fixed connective tissue cells. One must apparently concede that the latter can not only lay down both white fibers and elastic fibers, but also, under certain environmental stimuli, can show a metaplasia and assume an osteoblastic function. The only difference between the assumptions for the two groups of cells is that in the fixed connective tissue cell the variation in function is manifested in a variety of extracellular products, while in the wandering blood cells it is indicated by an intracellular variation. It seems quite possible then that one may have to assume for the whole mesenchyme group of cells a greater plasticity or a more permanent possession of embryonic characters than is generally assumed or than is shown by epithelial structures. If the transformation of smooth muscle into the striated voluntary muscle type as claimed by Carey under certain experimental conditions is confirmed, we have further evidence of plasticity even in the more fixed tissues of the middle germ layer.

The cell for which this totipotentiality is claimed has received at the hands of hematologists a great variety of designations; the large lymphocyte, the indifferent lymphoid cell, the lymphoblast, the lymphogonien, the myeloblast, etc. It is a cell with large, pale-staining, vesicular nucleus and a scant rim of basophilic protoplasm without specific

granulation—a young-looking undifferentiated cell. Such a cell is found constantly in the bone marrow and in lymphoid tissue. That a totipotential cell of this character is present, in small numbers at least, in the normal circulating blood appears to be the simplest explanation of such phenomena as the occurrence of red marrow in metaplastic cancellous bone in the aorta, as described by the author, and the experimental production of red marrow in association with metaplastic bone in the necrotic kidney of the rabbit as described by Maximow. Yet such a circulating cell has not been positively identified unless one assumes with Maximow that any of the smaller and larger lymphocytes may grow into this indifferent lymphoid cell and that this latter may then show metaplastic development. I have been inclined to regard the smallest lymphocytes as end cells and to assign embryonic characters to the large mononuclear cell with round nucleus and very basophilic protoplasm.

In certain pathological blood pictures and, in particular, in the acute leukemias of marrow origin, the circulation is fairly flooded with primitive cells. One has in the acute myelogenous (myeloblastic) leukemia an unlimited proliferation of this primitive cell without differentiation. Accordingly, there is lacking in the circulation every element that might be derived from it. Red blood cells show a constantly diminishing number (an aplastic anemia); granular leukocytes and blood platelets are lacking. Only myeloblasts and lymphocytes represent the leukocytic content. In the chronic splenomyelogenous (myelocytic) leukemia one has an equally unlimited proliferation but with partial differentiation, and the blood stream is flooded with myelocytes, nucleated red blood cells, platelets (small and large) and even with the megalakaryocytes. By contrast again, in pernicious anemia differentiation is toward the hemoglobin-containing cells, and marrow leukocytes and platelets show diminishing numbers as the disease progresses. I am aware that the "crowding out" theory has been advanced to explain the phenomena just cited, but it seems less satisfactory. The monophyletic theory seems eminently satisfactory even though it offers some difficulties in addition to the lack of absolute proof. Danchakoff objects that indifferent cells in marrow tissue should be subject to the same stimuli and thus differentiation should proceed in the same direction in all. I doubt the validity of the premise upon which the objection rests. Evidence tends much more toward the view that physical and chemical conditions are not uniform throughout even small masses of tissue and probably not throughout even the

colloid mass which constitutes the body of a single cell. Varying distances from the source vary the intensity of normal and toxic stimuli, oxygen tension and the like.

The assumption then of the totipotential cell simplifies the consideration of hematological problems if one can avoid the complicated schemes of development and relationship propounded by certain advocates of the monophyletic theory, notably Pappenheim. From this indifferent cell there is differentiated, as I have suggested elsewhere, a sufficient amount of varied marrow and lymphoid tissue in the body to maintain a fairly constant circulating number of red and white cells. Production keeps pace with destruction, as is shown by the constant ratio manifested in the circulating blood. While there is a rather wide variation in the normal number of leukocytes and the percentage of different forms as seen in the differential count in different people, for an individual in health the number and the differential picture are fairly constant. One may still say the average normal total leukocyte count is 7500 per cu. mm. (7300, Miller's figure for 280 cases). However, the author was surprised, in making differential counts on supposedly normal students at Wisconsin University, to find the proportion of the various forms of cells quite different from that given by Ehrlich. The 70 to 72 per cent of neutrophile leukocytes of the Ehrlich data was strikingly above the average given in the series of the author's counts (54.6 per cent) in which a Romanowsky type stain was used instead of the Ehrlich triple stain. Miller's repetition of the work on a much larger scale gives an intermediate figure of 62 per cent, in counts with Wilson's stain. I am inclined to accept Miller's figure not only in view of his larger material, but also as a result of my long-continued study of the changes produced in the leukocytic formula by low grade chronic non-disabling infections, particularly of the upper respiratory tract which are particularly prevalent in the locality in which my counts were made. Latent "childhood" tuberculous infection is quite apparently the modifying agent in some of the counts given.

The occurrence of a normal individual leukocyte formula raises immediately the question of its importance. Has it physiological significance and if so what significance? In answer to the question, it would seem that at the present time we have no evidence of what might be called a purely physiological function for the leukocytes. As far as it is known, their function may be said to be pathological. At least it is protective, and less physiologically protective than that of

skin. It is rather difficult to accept this conclusion when one considers that the leukocyte is a much more primitive cell than the physiological red cell, and appears much lower in the animal scale than the latter. Yet one must recall that protection of the body from attack and invasion by alien and enemy forms is needed by the invertebrate as well as by the vertebrate animal. In man and the higher animals all the exposed surfaces, inner as well as outer, are crowded with organisms, many of which are pathogenic and capable of invading the body or harming it by toxin production. Finding evidence in certain acute infectious diseases (measles, influenza) that pyogenic complications occur commonly when the total number of polymorphonuclear neutrophile leukocytes falls below a certain level per unit volume of blood, it has seemed justifiable to conclude that their number per unit volume is normally maintained at a protective level against these same pyogenic organisms present on mucous membranes. Bunting and Huston have described in normal animals the daily emigration of billions of lymphocytes upon the mucous membranes and in particular upon that of the intestine. It would seem that they fulfil their functions there normally. As the lymphocytes are not phagocytic it is suggested they act as affixers of toxins, and thus contribute to the immunity which the intestine has to the contained organisms. Limiting the function of these leukocytes to that of protection may be simply the expression of ignorance, yet it would appear to measure up to the present knowledge we have concerning them.

For further consideration of the leukocytes it would appear best to take up each type of cell separately.

The neutrophile leukocyte. Neutrophile leukocytes are found in the marrow of the human fetus between the fourth and sixth month. They are also found in connective tissue elsewhere, especially about the thymus and in the liver (Browning). In the sheep, Goodall finds an occasional leukocyte in the liver in the 2 to 3 cm. embryo; in the 4 to 5 cm. embryo leukocytes are fairly numerous about the thymus; above 5 cm. there is beginning hematopoiesis in the marrow which becomes active at 10 cm. After birth, leukocyte formation in the higher animals occurs normally only in the marrow. The mature neutrophile, with its characteristic nucleus, is a development through well-recognized changes from the myelocyte with round vesicular nucleus and a neutrophilic granulation. The myelocytes arise both homoplastically by mitosis in similar granular cells, and heteroplastically by the development of granules in a primitive cell with hyalin

protoplasm. I have emphasized the fact that the marrow cells are grouped in extravascular centers, in which the cells develop from the center toward the periphery, so that the mature cell comes to lie on the outside, adjacent to venous sinuses. The mature neutrophile, which is the most actively amoeboid of the circulating cells, apparently enters the blood stream through its active efforts and under some unknown chemiotactic influence. Equally unknown is the regulatory mechanism of the supply to the circulation. A normal marrow apparently contains a considerable supply of mature leukocytes. This is seen in sections of red marrow. It is also indicated by unpublished experiments in this laboratory which show that in the rabbit so slight a stimulus as the injection of 1 cc. of normal salt solution in an ear vein will call out a total of 300,000,000 neutrophiles (pseudo-eosinophiles) in one hour. This time interval is too short for the increase in circulating cells to represent other than an increased out-pouring. It is not apparent why but a certain proportion of these cells enters the blood stream normally and the rest remain in the marrow as a reserve.

As the neutrophile enters the blood stream its nucleus usually shows three lobes with connecting chromatin bands. Apparently as a result of ageing, the lobes increase in number to 4, then to 5 and even to 6, as emphasized by Arneth, who has constructed definite formulae, indicating the degree of marrow activity. The length of life of the neutrophile within the circulation and its ultimate fate have not been absolutely determined; Weiskotten and Steensland, by argument not entirely free from criticism, have concluded from leukocyte curves obtained in benzol poisoning in the rabbit, that the neutrophile lives 3 days in the circulation.

The granules in the protoplasm of the neutrophile are held to be endogenous and the result of protoplasmic activity, even by Weidenreich, and they are the only leukocytic granule so accepted by him. That any feature of its functions is displayed while in the normal circulation we have, as stated earlier, no evidence. The part played by the cell in pathological processes, in the coagulation of blood, in the digestion of proteid by its proteolytic enzyme, active in an alkaline medium (Opie), and in the ingestion and destruction of bacteria, in particular of the so-called pyogenic group, is too well known to need more than mention. It may be said however that it is becoming more evident that the neutrophile is not an active defensive agent against all infecting organisms, but that in several acute infections, notably typhoid fever, measles and influenza, and in at least one chronic infection,

tuberculosis, there is a marrow inhibition, with a diminished number of neutrophiles in the circulation. Defense seems to lie with the lymphoid and mononuclear groups of cells. Even in pyogenic infections, the endothelial phagocyte is found to play a part almost if not quite as important as that of the neutrophile.

The eosinophile leukocyte. Eosinophile leukocytes are found in the fetal tissues as early and possibly earlier than the neutrophile. Browning found them in the walls of the hepatic and umbilical veins in a fetus of 10 weeks and in the marrow at $3\frac{1}{2}$ months. The eosinophile of the adult circulation is a cell of marrow parentage, and like the neutrophile is descended through a series of myelocytes which may have had either a homoplastic or a heteroplastic origin. The theory championed by Weidenreich among many others that the eosinophile granule represents not an endogenous formation but exogenous material related to hemoglobin or its dissociation products, seems so far removed, as even a plausible deduction from the easiest observations and experiments, as to be an absurdity. The whole question of the eosinophile has been recently reviewed by Downey and by Ringoen, who give abundant proof of the opposing theory, if it were needed. With the early disposal of the theory that the eosinophile granule was but a later stage of the neutrophile, and with the demonstration of the endogenous nature of the granule one may recognize in the eosinophile a cell of specific character. Whether or not all eosinophiles are of marrow origin must apparently remain a question still to be discussed and investigated. Ringoen, apparently, is convinced that eosinophiles are formed in the hemo-lymph nodes of normal sheep, as well as in the marrow. In a study of hundreds of human lymph nodes I have never seen evidence of eosinophile production in them. Emigration of eosinophiles from the blood stream into lymph nodes is of extremely common occurrence, especially in pathological conditions, in which there is a destruction of lymphocytes within a node.

The function of the eosinophile cell is problematical. It is ordinarily not phagocytic. While its number in the normal circulation is small, under stimulation of the marrow and reaction of the latter the number in the blood may be augmented more than a hundred fold, with every evidence of emigration of vast numbers from the circulation. The cell appears to share in the defense against various animal parasites. In addition it is found in the tissues, in inflammations of the skin and mucous membranes of varied etiology and in the neighborhood of tumors of great variety. Eosinophilia is one of the phenomena of

anaphylactic shock. Ringoen sees a possible chemiotactic influence in the products of autolysis of cells as suggested by Fessinger, as an explanation for the appearance of the cell under this great variety of circumstances.

The basophile. The basophilic cell of the blood was at first designated a "mast cell" by Ehrlich on the supposition that it was identical with the "mast cell" of the tissues. The work of Maximow, Weidenreich, Pappenheim and others has shown such marked differences between the two cells that they must be recognized as of two distinct orders with a basophilic (metachromatic) reaction to granules in their protoplasm as the only common feature. According to Maximow there is to some extent a ratio between the number of basophiles in the blood and that of the tissue mast cells. Animals with many circulating basophiles have few in the tissues and the contrary is also true. The status of the circulating basophile has apparently not been definitely agreed upon. Pappenheim, Weidenreich and others consider it not a true granulocyte but a degenerating cell, the granules representing products of degeneration. Maximow and Downey, among others, are in accord with the original Ehrlich conception that it represents a specific granular cell of bone marrow origin. They describe homoplastic and heteroplastic development of basophilic myelocytes from which the circulating cell is derived. Physiological observations seem to confirm the latter point of view which was arrived at by the authors mentioned from a study of tissue sections. Circulating basophile leukocytes are increased relatively and absolutely in at least two conditions in which one has evidence of increased marrow activity, chronic myelocytic leukemia, and polycythemia vera. They are also increased in chronic inflammations of the accessory nasal sinuses. It would seem that they must be regarded as specific cells; but we are totally in the dark as to their function.

The large mononuclear and transitional of Ehrlich. These largest cells of the circulation, the so-called large mononuclear leukocytes or monocytes have been the subjects of much discussion relating to their origin, to their relation to other cells, and to their function. In the circulating blood we find, normally, large cells with two types of nuclei. The one has a large round or oval nucleus and a very basophilic protoplasm and forms in the author's large series of differential counts upon human blood, but 0.2 per cent of the circulating leukocytes, upon the average. The other has a nucleus of varied shape, at times merely indented, more commonly trilobed, at times S-shaped, and

there is an abundant protoplasm somewhat basophilic and containing fine azurophile granules with Romanowsky-type stains. This cell is more numerous than the former and constitutes approximately 6 per cent of the circulating leukocytes. It is this cell to which Ehrlich gave the name "transitional" on the mistaken theory that it represented a stage in the development of the neutrophile leukocyte. Ehrlich was uncertain whether its origin was from the spleen or bone-marrow but considered the latter most probable. For many years this cell was considered to be the makrophage of Metchnikoff, and thus concerned in phagocytosis. Mallory and his students have spoken of it as the "endothelial leukocyte" on the supposition that it is identical with the phagocytic endothelial cells freed from the lymph sinuses and hem-vascular walls. In her recent article in these REVIEWS Sabin practically accepts this view as a result of her investigation of the growing vessels in the yolk-sac of the chick. She finds multiplication of endothelial cells which wander both into the lumen of the vessel (monocytes) and into the tissues (clasmatocytes). In adult animals there is also shown to be multiplication of endothelial cells and their separation from the vessel wall and entrance into the circulation (Evans, McJunkin, Simpson et al). There is no doubt that under proper stimulation experimentally or under certain pathological conditions endothelial cells may be found in the circulating blood. Simpson in Evans' laboratory has shown that with the repeated injection of colloidal dyes into animals these cells occur in showers and may constitute 90 per cent of the leukocytes in the right heart, yet at the same time constitute but 0.1 per cent on the left side. Yet this is not proof, as some authors seem to contend, that endothelial makrophages and large mononuclears are identical.

The application of vital staining to the leukocytes, both intravitam and on the slide (supravital method), it has been hoped would settle this question. It is perhaps unfair to depend entirely upon a so-called biological method to determine cell-relationship. Because two cells ingest or absorb the same type of dye-stuff would not seem an absolute proof of identity. It can be shown experimentally that both endothelial cells and polymorphonuclear neutrophiles will engulf staphylococci. They are thus far biologically related. Likewise large mononuclears and endothelial cells will behave alike toward certain dyestuffs, differently toward others. The results and interpretation of results vary in different hands. Without pursuing the controversy at length, I think the fairest statement of what the present day point of view

must be, is the conclusion of Miriam Simpson, that while her extensive work with vital stains "points strongly to the biological affinity of these two interesting cell types, it cannot be said to show an identity or that one may be transformed into the other in the free or circulating blood stream. This evidence is in consonance with what we know about a similar lack of transformation of one type of leukocyte to another in the blood current, our information tending to bring the conviction that a cell once shed into the stream henceforth usually undergoes no significant transformation."

My own study of the leukocytes of the circulation in normal and pathological states has led to the conclusion that the large mononuclear with the round nucleus is an immature cell and is found in the normal circulation largely as an accident. In its mature form it is the transitional cell of Ehrlich. The cell of this type, found normally, is a marrow cell and is increased in marrow reactions of a proliferative character. Yet in certain pathological conditions with unusual stimulation of the lymphoid tissue all the large mononuclears found in the blood are not of this origin. The large mononuclear group becomes heterogeneous and includes many cells of lymph gland origin. This is true not only of the acute lymphatic leukemia picture, but also of the blood in such infections as smallpox and measles. Endothelial cells may also be found in the blood in these diseases.

The lymphocyte. It is quite generally agreed that most, if not all the lymphocytes of the circulating blood are derived from the lymphoid tissue in the body and reach the circulation through the large lymph channels. This would seem abundantly proved by the work of Rous, Carlson and Davis, and Bunting and Huston. I find, however, as late as 1908 Gulland and Goodale expressing the opinion that most if not all of these cells of the circulation have their origin in the bone marrow. Although extramedullary lymphoid tissues furnish the main source of lymphocytes, it is generally accepted that typical areas of lymphoid tissue are found in the marrow, and that this tissue may furnish some cells to the circulation.

In the normal circulating blood one finds smaller and larger lymphocytes. The larger have the same type of nucleus, but this is surrounded by more protoplasm of a less basophilic reaction, containing usually more coarse granules. It seems safe to assume that this represents but an older stage of the smaller cell. It is not to be confused with a larger type of lymphocyte with very basophilic protoplasm which appears in the circulation under intense stimulation of the lymph glands

in certain infectious diseases, notably measles and smallpox, and also in the acute type of lymphogenous leukemia. This last cell is present in normal lymphoid tissue but does not normally appear in the circulation. Certain unpublished observations of the author and Mr. Huston show that if one makes impression preparations from lymph glands of the rabbit and measures the cells, while all gradations are found, the greatest number of the cells group themselves into three major groups; the largest cells, corresponding to the lymphoblast, have a diameter of from 18 to 20 μ; an intermediate group which varies between 10 and 12 μ; and the groups of smallest cells between 5 and 7 μ. Mitotic figures were found in cells of the first two groups, but were not seen in the smallest cells. This suggests a development similar to that of the red cell in which the intermediate red cell of Howell stands between the megaloblast and the normoblast.

It is generally accepted that it is by mitosis in the lymphoblasts of the germinal centers of the lymph glands that the development of the lymphocyte is inaugurated. This intermediate sized cell with its capability of mitotic division and thus of a geometric progression in the multiplication of cells, has not been generally recognized. It may, however, account for the undue number of small lymphocytes apparently produced by mitosis in a few lymphoblasts.

There is still uncertainty as to the embryology of the lymphocyte and even of the post-natal origin of the cell. Some, as Danchakoff, maintain the development of the lymphocyte from a hemocytoblast, the primitive blood cell cut off from the mesenchyme. Others, as Thiel and Downey, maintain that small lymphocytes are cut off directly from the mesenchyme cells without the intervening hemocytoblast stage. Sabin's study of the lymph nodes indicates the possibility of this latter mode of development without, in her opinion, proving it. Weidenreich and Downey hold it definitely proved that in post-natal life, the reticulum of the lymph glands, the fibroblastic tissue of the omentum, and possibly also that of the loose connective tissue retain the capactiy of the embryonic mesenchyme to liberate free lymphoid cells. The orthodox view holds the lymphoblast of the germinal center as the parent of the lymphocyte in the adult animal. The necessity of postulating a tissue lymphocyte has largely departed with the general recognition of amoeboid motion in the lymphoid cell, yet complete harmony of views on the origin and relation of the cell cannot be expected at the present state of our knowledge.

The work of Rous, Carlson and Davis, and others has shown that more lymphocytes enter the blood stream during 24 hours than are present in the circulation at any one time. Bunting and Huston, in searching for a solution as to what becomes of this large number of cells in normal animals, have found that they not only leave the blood stream but also the body finding their way out onto the mucous membranes and in particular onto the intestinal. Their normal function is apparently fulfilled there. This function is unknown. If it be protective against the bacterial content of the intestine, it cannot be of a phagocytic nature, as the lymphocyte is not a phagocyte. It is suggested that it may be antitoxic or, in other words, that the lymphocyte may contribute to the immunity of the intestine to its contained bacteria by affixing toxins. During the recent years the lymphocyte has gained increasing consideration as a defense cell in pathological processes. The work of Murphy and his associates has demonstrated that it is an important cell in the defense of the body in such infections as tuberculosis and against cancer. The nature of this defense, again, is unknown. Owing to its lack of phagocytic power one seems left with but the possibility that the lymphocyte acts by affixing toxins and if not destroyed by that toxin, by the production of antibodies. The normal distribution of lymphoid tissue in the body, the position of lymphoid cells in pathological tissues all speak for such a theory.

It seems unnecessary to further condense this inadequate summary of hematological problems into a formal set of conclusions. In the present state of our knowledge, judgment passed on many of these problems would be premature and would have only the weight of opinion. It must be left to further investigations to determine in full the life history and the functions of the different leukocyte forms.

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THE PRESENT STATUS OF THE FUNCTIONS OF THE THYROID GLAND

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We probably possess a more detailed knowledge of the embryology, anatomy, physiology, chemistry and pathology of the thyroid than of any other gland. But as regards its interrelations with other tissues,—a vastly greater and more important field,—it can truthfully be stated we are only at the beginning of any definite knowledge.

No attempt will be made to refer to or review all the literature relative to thyroid physiology that has appeared during the last decade. Excellent reviews of this type are available in an article by Vincent (1) in 1911 and in certain textbooks, notably Biedl's (2). Rather a critical summary of the present status of certain phases of our knowledge of this gland and some of the important steps involved in acquiring it as they appear to the author will be attempted. Owing to the recent great popular interest in endocrinology (one is tempted to say endocriminology) the thyroid gland has suffered with the rest, though perhaps less than its sister glands, from loose speculation. This, however, is only because we possess a greater number of unchallengeable facts relative to the thyroid.

ANATOMY. *a. Ancestry and embryology.* Owing to the survival of one class of vertebrates—the Cyclostomes—it has been possible to establish the ancestry of the thyroid of all higher vertebrates as a direct metamorphosis of the endostyle organ. The endostyle organ is an elaborate ventral mid-line pharyngeal gland in Tunicates, Amphioxus and Ammocoetes (3). In Tunicates and Amphioxus it opens into the pharynx through a groove extending the full length of the organ (5). In Ammocoetes the opening into the pharynx is reduced to a large duct (4). During the metamorphosis of Ammocoetes the endostyle organ undergoes atrophy with complete loss of three of its specialized types of epithelium including the duct, and the ductless thyroid follicles of the adult are formed from one persisting type of endostyle epithelium (6). These cells sometimes retain their cilia after metamorphosis. The ductless thryoid follicles in Cyclostomes arise solely from the endostyle

organ. Studies in the embryology of the thyroid of fishes, amphibians, reptiles and birds have shown that it also arises solely from a median single ventral tubular downgrowth of the pharyngeal endoderm in or slightly anterior to the first aortic arch (8), (9). This symmetry and uniformity of development was believed to be departed from in mammals owing to the discovery by Stieda (10) of the so-called lateral thyroid anlagen. These lateral thyroid anlagen were believed to give rise to the lateral thyroid lobes and to be developed from the fourth gill pouches, or more accurately in man from the rudimentary fifth. The view that the thyroid in mammals arises from three separate anlagen persists and still appears in many textbooks. Recent studies of the fate of the so-called lateral thyroid anlagen, post-branchial bodies, or better, ultimo branchial bodies, show that they are only atrophic remnants from the fifth pair of gill pouches which, during development, may become attached to or even embedded in the lateral thyroid lobes, but take no part in the formation of thyroid tissue (11), (12). This solution of the origin of the mammalian thyroid makes it possible to interpret many of the pathological changes and developmental defects of the thyroid, and makes the origin and development of this gland uniform throughout all vertebrates (7), (13).

b. Gross and microscopic. Morphologically the thyroid is one of the simplest of body tissues and resembles the lung more closely than any other tissue. Indeed, there are many embryological, anatomical physiological and pathological relations between the thyroid and the lungs the study of which has added much to our present-day conception of interrelationships. The thyroid tissue is one of the most labile tissues in the body—capable of rapid overgrowth and of equally rapid involution. Its wide range or cycle of morphological changes makes it possible to detect easily morphological changes which if interpreted in terms of function are but little removed from the normal, but if compared with similar types of morphological changes in other tissues less well endowed with the capacity for growth have frequently resulted in drawing wholly unjustified conclusions regarding the alteration of function.

The thyroid has only one known cycle of cell changes and it tends to repeat this cycle in response to all stimuli involving increased functional activity.

The normal human thyroid weighs between 20 and 25 grams and does not exceed 0.35 gram per kilo of body weight (36), (14). Statistical data indicate that the thyroid is slightly larger in females per unit

of body weight. The gross outline of the thyroid is quite variable, the greatest variation occurring in the isthmus or pyramidal process (16). In the strictly normal human thyroid the isthmus is a band of tissue from 1 to 2 cm. in width and from ½ to 1 cm. in thickness connecting the two lateral lobes across the trachea anteriorly just below the level of the cricoid cartilage. The presence of a pyramidal process and thyroglossal stalk must be considered as due to incomplete absorption of this portion of the thyroid tract which normally takes place between the fifth and eighth week of fetal life. In severe endemic goiter districts approximately 95 per cent of human thyroids have well-formed pyramidal processes and thyroglossal stalks frequently continuous with the foramen cecum (15). Similar variations are seen in animals; thus, in congenital goiter of dogs and sheep the thyroid lobes are usually joined by an isthmus while normally in these animals the isthmus undergoes absorption before birth.

The gland is invested with an outer fascia which strips readily and exposes a slightly lobulated smooth surface formed by the inner or true capsule. This is thin and translucent. Thickened portions of this capsule (trabeculae) extend into the gland, support the blood and lymph vessels and give it a slightly lobulated appearance (34), (35). The color of the normal thyroid varies from a pale translucent amber red to a bright translucent amber red. The normal gland is of firm consistency and made up of closely packed circular or oval closed alveoli filled with a glairy honey-colored viscid globulin—the so-called colloid, which gives to the thyroid its specific characteristic, chemical as well as physical. The thyroid unit or alveolus is similar in all vertebrates (30), (31), (32). In man these alveoli vary from 0.1 to 0.5 mm. in diameter and are lined with a single layer of low cuboidal epithelium (columnar epithelium always indicates hypertrophy) (17). The older observers (Langendorf (18), Biondi (19), Hürthle (20) and others) recognized two types of gland cell, the so-called chief and colloid cells. The former are more granular while the latter contain in addition vacuole-like globules filled with a thin fluid which some have considered as a thyroid colloid, similar in many respects to that contained in the alveolar spaces (33).

In recent years a great deal of attention has been paid to the finer specialized cytoplastic structures especially the mitochondria and the reticular material or Golgi apparatus (27), (28), (29). Cowdry (21), (22), (24) has reviewed the literature of both these subjects. Mitochondria (Altmann's granules) are present in all thyroids. They are

very rare in the fetal thyroid and increase with age (23). Their number also varies with the size of the cell. Their lipoidal nature distinguishes them from secretion granules with which they were long confused. They can be correlated with functional activity as well as with cell growth. Bensley noted that the more active the cell the more numerous were its granules. Some authors, notably Goetsch (25), have in addition attempted to establish a relation between the pharmacological activity of the cell's secretion and mitochondria, particularly in exophthalmic goiter. This view has not received much support for the reason that the very actively hyperplastic columnar cells seen in the thyroids of myxedema are equally rich in mitochondria. They seem closely related in the functional activity of the cell but unrelated to the pharmacological value of the cell's secretion. In the thyroid cell, mitochondria are most numerous in the region between the lumen and the nucleus while in ordinary gland cells like the salivary or pancreas these granules are most numerous in the region between the nucleus and the basement membrane. Bensley pointed out that this was probably due to reversed polarity. Recently Cowdry has studied the thyroid cells of the guinea pig, using the reticular material or Golgi apparatus as an indicator of polarity. He found that the reticular material of the guinea pig thyroid cell was located in the region between the nucleus and the base of the cell in about one cell out of every five hundred, i.e., reversed, and accepts Bensley's (26) explanation that it indicates the ability of the thyroid to secrete either toward the bloodstream or toward the alveolar lumen, and this reversed secretion (toward the lumen) comes into play when the thyroid secretion is being produced in excess of the body needs. Reversed polarity has been observed only in the thyroid gland and as the ancestral thyroid (endostyle organ) was an external secreting organ it suggests that the reversed polarity is a relatively recently acquired characteristic to meet a change in function.

Lymphoid tissue, represented by scattered small foci in the stroma, is normally present in the thyroid (37). Under certain conditions associated with general overgrowth or persistence of lympoid tissue, as in status lymphaticus, Addison's disease and exophthalmic goiter, this lymphoid tissue may undergo an extraordinary hyperplasia with the development of well-formed germinal centers.

Accessory thyroid tissue other than that found along the thyroglossal tract occurs with great frequency. In dogs and cats accessory masses may be demonstrated in upwards of 90 per cent. The most frequent

sites are in the thymus gland and in the region of the arch of the aorta.

In man there are normally small groups of undeveloped thyroid cells lying in the stroma between the alveoli. These have been spoken of as cell rests and their relation to the development of thyroid adenomata was first pointed out by Wölffler (43). It is believed that the thyroid anlage is capable of and actually produces during embryonic life many more cells than are usually required for functional needs. Under ordinary conditions, as in the case of striped muscle fibers during fetal life these thyroid cell rests undergo gradual absorption beginning in intrauterine life and continuing after birth. On the other hand, in the presence of a stimulus for increased thyroid activity, these undeveloped rests respond with growth and become the adenomata which are almost universally present in and an integral part of endemic human goiter. Thyroid adenomata are exceedingly rare in the lower animals.

These adenomata are highly variable in size, number and structure. One recognizes types of adenoma composed of closely packed small undistended alveoli—so-called fetal adenoma, and also types with well differentiated large colloid-containing alveoli and finally all gradations between these. Morphologically different types may be present in the same gland. It is believed these variations in morphology depend upon *a*, the stage of differentiation of the cell rest from which the adenoma arose; and *b*, the degree of differentiation occurring during its growth and involution. Adenomata are capable of taking up iodin from the circulation and of elaborating the iodin-containing hormone, thus differing sharply from thyroid carcinoma. The more differentiated types as regards evidence of functional activity approach that of normal thyroid while the fetal types may or may not be able to take up iodin even after the prolonged administration of iodides (44), (45).

c. Circulation. The thyroid has a very large blood supply variously estimated from 3.5 to 5.9 cc. per gram per minute (66), (67). Obviously in a tissue with such wide variations in functional activity correspondingly wide variations in the blood supply must be present. Control studies should be made with standardized thyroid and the thyroid happens to be the only tissue which at present can be standardized. This is accomplished by iodin administration. The thyroid arteries form rich anastomoses on the surface but none in the depths of the gland (40). Schmidt (38) and Horn (39) have described endothelial buds or "knospen" in the arterioles which may act to reduce the speed of the blood flow by a baffle-board effect. This also reduces the pulse-wave effects which in a gland with such a short and wide capillary

path would otherwise easily pass through to the veins, especially in the marked hyperplasias. The thyroid gland can be perfused readily at a pressure of 15 to 20 mm. Hg. while the kidney requires from 70 to 80 mm. Hg. pressure. The capillary network surrounding each alveolus is comparable to the capillary network surrounding the lung alveolus. The thyroid is also richly supplied with lymphatic vessels which are collected for the most part into two major channels which leave the gland with the large veins (41). The thyroid hormone can pass directly into the blood stream, as has been shown by Rogoff and Goldblatt (42). There is no evidence except the older morphological evidence that it may also be discharged into the lymphatics.

d. Innervation. The anatomical studies of Berkeley (46), Anderson (47), Rhinehart (48) and others have shown that the thyroid is richly supplied with nerves, all of which are believed to belong to the sympathetic system. These nerves leave the spinal cord between the second and seventh thoracic segments and pass upward to the middle and superior cervical ganglia from whence they are relayed to the thyroid *a*, directly along the blood vessels, or *b*, indirectly through the superior laryngeal and possibly the recurrent laryngeal nerve. These nerves are distributed both to the perivascular tissues and to the bases of the gland cells—the latter have been recorded by anatomists as possible secretory nerves (56), (56a). The gland is richly supplied with vasomotor nerves both constrictor and dilator (54), (55). The question of secretory nerves has attracted much attention during the last decade and still remains undecided. All the evidence is indirect. Asher and Flack (49), (50) first showed that in rabbits the blood pressure response to a given dose of adrenalin was greater after electrical stimulation of the superior laryngeal nerve with intact thyroid than before such stimulation. They also showed that after thyroid removal, stimulation of the superior laryngeal nerve did not modify the blood pressure response to a similar dose of adrenalin. Oswald (51) obtained similar results by injecting iodothyreoglobulin instead of stimulating the thyroid nerves. Levy (52) has confirmed Asher and Flack's work on cats. Epinephrin produces all these effects and also markedly raises the heat production in the absence of the thyroid gland. The difference is only one of degree. Von Cyon (53) has criticized Asher and Flack's work by pointing out that where blood pressure is used as an indicator, many factors particularly the degree of anesthesia must be carefully controlled, and also one must distinguish between goiterous and non-goiterous rabbits for this kind of work. Rahe, Rogers, Fawcett, Beebe and others

(58), (59) have shown that the iodin content of the thyroid may be reduced by prolonged faradic stimulation of the cervical sympathetic. Cannon and Cattell (57) showed that there was an alteration in the electrical potential of the gland on stimulating the cervical sympathetic. No such effect was observed when the vagus or sciatic nerves were stimulated or when the systemic blood pressure was changed. They interpreted this as evidence of secretory innervation.

All these methods are highly indirect and complicated and the results are not susceptible of complete analysis at present. Other more direct proof must be offered before one can accept the doctrine of secretory nerve control of the thyroid secretion. That some sort of nervous control whether secretory or regulatory exists is probable on the grounds of analogy. Whether this regulation is dependent on specific nerves or on specific chemical changes acting through a more general regulatory nervous mechanism is still to be demonstrated. On the other hand, there is very direct evidence that specific nerve endings are not necessary for thyroid tissues to show many evidences of variation in secretory activity. Thus, by autografting the thyroid in widely separated parts of the body it has been shown that such transplanted thyroid wherever located shows chemical and morphological changes paralleling those of the non-transplanted tissue (60). If the transplant is undergoing hyperplasia, the non-transplanted portion is also undergoing changes similar in nature and degree. The administration of iodin inhibits hypertrophy alike in the transplanted and non-transplanted thyroid. In 1914 Cannon (61), utilizing Langley and Anderson's (62) method of anastomosing motor and sympathetic nerves, united the anterior root of the phrenic with the peripheral end of the cervical sympathetic in the cat and reported the occurrence of symptoms closely resembling exophthalmic goiter—tachycardia, emaciation, increase in metabolism to plus 100 per cent. He ascribed these phenomena to the bombardment of the thyroid with impulses discharged through the phrenic stump. These results have neither been confirmed by others (63), (64), (65) nor repeated by Cannon.

PHYSIOLOGY. *a. Effects of thyroid removal.* Our knowledge of the physiology of the thyroid may be said to have begun with Gull's report in 1874, *On a Cretinoid State Supervening in Adult Life in Women* (74). Prior to its publication, the function of the thyroid was either speculative—that it aided in the formation of erythrocytes, that it acted as the vascular shunt for the cerebral circulation, that it neutralized toxins, that it served only to give form to the neck, etc.,—or was hopelessly

confused with the function of the parathyroids. It was not until Gley in 1891 (75) rediscovered the parathyroids that any real differentiation of the functions of the thyroid and parathyroids was possible, and it was some twenty years after this that their functions were finally separated to the satisfaction of all workers.

Independent observations reported by the Brothers Reverdin in 1882 (76), and more clearly by T. Kocher in 1883 (77), on the effects of total thyroidectomy in man for goiter established the first experimental confirmation of Gull's clinical-pathological observations. Some of these operated human cases developed parathyroid tetany and died, while others developed during the next thirty to sixty days a cachexia which these surgeons recognized as similar to that described by Gull. The Reverdins designated the symptoms complex as operative myxedema (Ord (78) in 1878 having given the name myxedema to the condition described by Gull because his chemical examinations indicated there was an increased mucin formation in the thickened subcutaneous tissue) while Kocher called it *cachexia strumapriva*. Horsley (79), (80) in 1884, working with monkeys, observed a few instances of cachexia strumapriva; most of the monkeys developed tetany. Other observers carried out similar experiments on rabbits, sheep, goats, dogs, cats and guinea pigs. Those working with rabbits claimed that thyroidectomy was usually without noteworthy effect, while those working with carnivora usually obtained parathyroid tetany. The experimental thyroidectomies before 1891 in general added confusion rather than facts to the function of the thyroid. Since 1891 many species of mammals have been subjected to thyroidectomy using standard surgical technique and excluding the parathyroid factor. The most striking effect of thyroidectomy is a reduction in the total metabolism. In cats and rabbits this decrease begins usually in from six to eight days after thyroidectomy and reaches its lowest level between the twentieth and thirtieth day (81), (82). This low level of metabolism may be maintained for years (rabbit) or as accessories develop the metabolism may gradually rise to normal. In these animals the average maximum reduction in metabolism is around 35 per cent to 40 per cent, which corresponds closely to that observed in the severest forms of human cretinism and myxedema and may be designated as the myxedema level.

While qualitatively the symptoms following thyroidectomy in both young and adult animals are similar, the visible manifestations are strikingly more prominent in animals thyroidectomized during the period of growth. Adult herbivora like the rabbit, sheep or goat may

show very little change clinically beyond a dryness and thickening in the skin, thinning of the hair, a gain in weight and a lowering of body temperature. This was observed by the earlier workers. Heat production measurements in these animals, however, show the usual marked decrease. In the young there are in addition the gross manifestations of stunted physical, mental and sexual development.

With a knowledge of the frequency and functional importance of accessory and aberrant thyroid tissue, the writer believes that in the adult animal (sheep, rabbit, cat) the thyroid is not essential for vegetative life, while in the young it is only indirectly essential in that it is necessary for growth and development. The thyroid is an organ acquired late in the development of animal life, present only in the higher Chordates, and all we know of its function indicates that it provides the means for maintaining a higher level of metabolism and for varying its rate.

b. Biochemistry. The next most important advance in thyroid physiology was the demonstration in 1891 by Murray (82a), of the remarkable therapeutic effect of the injection of a glycerol extract of fresh sheep's thyroid in cases of Gull's disease. This was quickly followed by the independent announcements in 1892 by Howitz (83), by Mackenzie (84) and by Fox (85), that thyroid either fresh or dried or boiled was equally efficacious when fed by mouth. Emminghaus and Reinhold (86) in 1894 showed that thyroid feeding also caused a marked reduction in the size of goiter. These discoveries of the therapeutic effects of thyroid feeding also mark the beginning of thyroid biochemistry. The names of Hutchinson (87), (88), (89) in Great Britain, Frankel (90) in Austria, Baumann, Roos (91), Oswald (92) and Dréchsel (93) are most closely associated with the biochemical work of this early period which culminated in the announcement in 1895 by Baumann (94), (95), (96) of Freiburg that iodin in a rather firm organic combination was a normal constituent of the mammalian thyroid. He obtained a substance by acid hydrolysis which he named "iodothyrine." This substance was later put on the market by a pharmaceutical firm under the name of "thyroidin." The substance obtained by Baumann was a brown amorphous powder insoluble in water and acids, slightly soluble in alkalies, gave no protein reactions, contained 0.4 to 0.5 per cent P. and as high as 9.3 per cent iodin. Later work has shown that this method of hydrolysis (10 per cent H_2SO_4) partially destroys the specific iodin compound. Baumann and his pupils, Roos and Goldman (97), showed that iodin was present in the

mammalian thyroid in very variable amounts and that feeding iodin increased the store. Oswald (98), (99), in 1899, observed that the iodin was contained in the colloid, and that the colloid of the thyroid was mainly globulin, and introduced the terms thyreoglobulin and iodothyreoglobulin. He showed that the iodin content varied in general with the amount of visible colloid in the glands, but that hyperplastic glands could be rich in globulin and iodin-free. The relation of iodin to the structure of the thyroid has been particularly studied by Marine and Williams (100) and Marine and Lenhart (101). These studies have cleared up the controversy that had developed concerning the fundamental importance of iodin in thyroid physiology, because many workers noting the absence of iodin in the thyroid in certain conditions still claimed it was only an accidental constituent probably excreted into the thyroid as a waste product. Much of the earlier work claiming the absence of iodin in the thyroid was, of course, due to faulty methods of chemical analysis. The final results in all of this work showed that the iodin store in the thyroid varies in general with the amount of stainable colloid, inversely with the degree of active hyperplasia and in the extreme degrees of active hyperplasia seen in cretinoid states in man and animals the iodin store may be entirely exhausted. In the following table the relation of iodin to histological structure as found by Marine and Lenhart are given:

	NORMAL	EARLY HYPERPLASTIC STAGE	MODERATE HYPERPLASTIC STAGE	MARKED HYPERPLASTIC STAGE	COLLOID OR RESTING STAGE
Man.....	2.17*	0.88	0.71	0.32	2.00
Dog.....	3.32	0.62	0.37	0.11	1.99
Sheep.....	2.47		0.40	0.01	3.00
Ox.....	3.46	1.65		0.19	
Pig.....	2.51	1.10			2.35

* Iodin in milligrams per gram dried gland.

Earlier workers reported discordant results regarding the presence of iodin in the fetal thyroid (102). Fenger (103), (104), using standard methods of analysis, showed conclusively in a large series of animals that iodin is present in the fetal thyroid of cattle, pigs, sheep and man. In cattle it is present as early as the third month of intra-uterine life, that is, six months before birth. The iodin content gradually rises with the increasing age of the fetus and shows the same variations dependent upon structure as seen in extra-uterine life. The writer has

made similar observations on a large series of fetal thyroids of dogs. The iodin store in the thyroid shows striking seasonal variations, being highest in the early autumn (October) and lowest in the early spring (April) in this latitude (Seidell) (105), (106). The thyroid has an extraordinary affinity for iodin (107), (108). This was first observed by Baumann in 1895, though iodin has been knowingly used in the treatment of goiter since 1820 (Coindet) and unknowingly throughout many parts of the world, civilized and uncivilized, for unknown centuries. Iodin given to pregnant mothers is also readily stored in the fetal thyroid. The amount of iodin taken up by a given thyroid varies with the degree of active thyroid hyperplasia. The maximum store per gram being relatively constant, for most mammals thus far examined averaging between 5 and 5.5 mgm. per gram of dried thyroid (109). The minimum amount necessary for the maintenance of normal gland structure is likewise relatively constant, averaging about 1 mgm. per gram of dried substance (110). The average normal iodin content for human thyroid is around 2 mgm. per gram of dried substance and the maximum total store of iodin in a strictly normal human thyroid does not exceed 25 mgm. (111), (112), (113), (114). These facts are of the utmost importance in the practical application of iodin to the prevention of goiter.

Perfused surviving thyroids show the same marked ability to take out and store iodin from the circulating fluid as is seen in the thyroid in situ (115), (116), (122). It has been shown that the iodin content of the thyroid of a dog may be increased several hundred per cent within five minutes after the injection of 50 mgm. of potassium iodid into the femoral vein. As much as 18.5 per cent of a single intake of 38 mgm. (50 mgm. KI) given to a dog by mouth may be recovered from the thyroid whose ratio to body weight was as 1:687. The thyroid stands alone at present among the specific affinities of tissues for inorganic substances.

The older literature contains many reports of the presence of appreciable amounts of iodin in the parathyroids, thymus, pituitary and other organs. Excluding its presence in tissue due to the recent administration or to contact with iodin, active normal tissues other than the thyroid do not contain amounts greater than could be accounted for by its discharge from the thyroid. A great deal of work has been done in the attempt both to isolate and to synthesize the active iodin compound of the thyroid, especially by Oswald (117), Nürnberg (118), Koch (119) and many others (120), (121). Whole proteins—casein, glutein, serum-

albumin and globulin and many amino acids including tyrosin, tryptophane, histidin and phenylanilin have been iodized but none of these were active. Later work, using the tadpole as a test object, showed that iodized blood serum, especially the globulin fraction, has a slight thyroid-like effect (123). In 1916 Kendall (124) reported the isolation of the specific iodin compound in crystalline form which he named "thyroxin." According to his latest report (125) this substance has the empirical formula $C_{11}H_{10}O_3NI_3$ and structurally is trihydro-triiodo-oxy-beta indoleproprionic acid. This substance in the purest form yet obtained contains 65 per cent iodin, has a melting point of around 250 and crystallizes in sheaves of needles. Kendall has shown that this substance produces the same pharmacological effects as whole thyroid. Kendall also showed that iodin is present in the thyroid in both an active and inactive form (126). Taking advantage of the extraordinary affinity of the thyroid for iodin and of the Gudernatsch tadpole test, Marine and Rogoff (127) carried out experiments to determine the rapidity of the production of active thyroid iodin. Differences in pharmacological activity of the thyroid were detectable in eight hours after intravenous injection of 50 mgm. KI and the differences in activity were quite marked after thirty hours. These observations indicate that while the storage of iodin is instantaneous the formation of thyroxin is a relatively slow process.

In 1895 Magnus-Levy (128), (129) reported in Gull's disease that the respiratory exchange was markedly decreased below normal and that in Graves' disease it was notably increased. He also demonstrated that feeding thyroid to cases of Gull's disease markedly increased their respiratory exchange and the excretion of urinary nitrogen. Friedreich Müller had recognized the increased nitrogen excretion in Graves' disease in 1893 (130). These discoveries by Magnus-Levy are the most important contributions to the pharmacology of thyroid substance and among the most important contributions to our knowledge of thyroid physiology. The most characteristic pharmacological action of thyroid or of its isolated active substance, thyroxin, is an increase in total metabolism (131), (132), (133), (134), (135), (136), (137). Its action is in general proportional to its iodin content as determined either by measurements of heat production, nitrogen excretion or the Gudernatsch tadpole test (138) (the most sensitive test yet developed for thyroid). In tadpoles thyroid substance causes a rapid loss in weight associated with metamorphosis in a few days. This is proportional to the active iodin (139). Some have considered that the action

of thyroid on tadpoles was a specific stimulus to differentiation. All the phenomena observed may be explained on the well-known action of the thyroid in accelerating metabolism and the apparent specificity does not depend on a new specific action of thyroid but on its application to a living organism at a specific period in its development (140). The acetonitrile test of Hunt and Seidell (141), (142), (143) is not specific for thyroid activity, since while thyroid feeding increases the resistance of white mice to acetonitrile poisoning it decreases it in rats and guinea pigs. Further, removal of the thyroid does not alter the response in mice and the blood of thyroidectomized animals also protects. In man, Plummer (144) has roughly estimated that for the normal individual approximately 1 mgm. of thyroxin daily is sufficient for normal metabolic activity.

The effect of thyroid on the heart and circulation has been particularly studied by von Fürth (145), (146), von Cyon (53) and Oswald (147). Aqueous extracts of the whole gland injected intravenously caused the usual lowering of blood pressure, while purified solution of iodothyreoglobulin causes only a slight lowering of blood pressure but the heart rate is notably increased after a latent period. Oswald believes that thyroid increases the irritability of all sympathetic nerve endings. The thyroid has no specific effect on blood coagulation. In Graves' disease the coagulation time is usually prolonged but attempts to establish a thyroid relation have been negative (148), (149), (150). The relation of the thyroid to immunity has received a great deal of attention and the literature is confusing and contradictory. In general it has been found that hemolysin and agglutinin formation are higher in thyroidectomized than in control rabbits, while antitoxin (diphtheria) formation is lower in thyroidectomized animals (dog, horse and rabbit) (151), (152), (153), (154), (158), (159), (160). Fjeldstadt (155) in eighteen thyroidectomized rabbits found no increase in agglutinin formation. Most observers have reported numerous exceptions to the above general statement except Ecker and Goldblatt (156) who found the hemolytic titer of thyroidectomized rabbits always higher than the controls. It is stated that anaphylactic shock does not occur in guinea pigs if sensitized after thyroidectomy but does occur if sensitized before thyroidectomy (157). At present the results obtained do not warrant any direct association of the thyroid with antibody formation. The reaction to infections as shown by a reduction in iodin store and a tendency to hypertrophy and hyperplasia clearly indicate the thyroid is an important indirect factor in resistance

to infections. The increased heat production in infections is to some extent dependent upon the thyroid.

c. Regeneration and transplantation. The mammalian and avian thyroid regenerates rapidly after partial removal. Two major factors—the amount removed and the administration or the withholding of iodin, and probably many minor factors—age, diet, species, determine the degree of regeneration (70). In the dog, if one removes three-fourths of the gland, ordinarily regeneration occurs in the remaining fourth, but if small amounts of iodin are given, such regeneration does not take place. If as much as nine-tenths of the gland is removed, iodin in any amount does not protect against regeneration. Halsted (68) made an extensive study of thyroid regeneration in 1889. Ribbert (69) showed that regeneration may begin within a few days after partial removal and occurs first in the sub-capsular zone. His suggestion that this centrifugal growth is dependent upon a more active blood supply is probably correct. The irregular insular hyperthrophy and hyperplasia often seen in human goiter may be thus explained. Anatomically and chemically the thyroid changes in regeneration are identical with those occurring in the spontaneous hyperplasias of simple goiter and are controllable by the same methods, i.e., cellular hypertrophy and hyperplasia do not occur until after the iodin store falls below a given level (0.1 mgm. per gm. dried) (70).

Transplantation of the thyroid has been extensively studied by Cristiani (71), L. Loeb (72) and his co-workers, and by Manley and Marine (73). Thyroid tissue autografts readily in any part of the body and shows all the chemical and morphological reactions seen in the non-transplanted tissue. Growth of the transplant varies inversely with the degree of thyroid insufficiency created in the host. The administration of iodin or desiccated thyroid inhibits the growth of thyroid transplants. In much of the older work on transplantation, attempts to transplant large pieces, even whole glands, were failures. As only the peripheral layer of not more than four to six cells in thickness survive, the ideal transplant is a slice of tissue about 50 microns in thickness laid on some flat surface, as the subcutaneous tissue or the sheaths of muscles. The frozen thyroid tissue of rabbits also transplants readily (Manley and Marine). Many such experiments were made where the tissue was frozen with carbon dioxide from one to five minutes. The temperatures reached were not measured. Homeografts are rarely permanent. Barring technical errors, they all "take" but begin to undergo absorption as early as the seventh or eighth day.

Some animals destroy initial homeografts much more slowly than this, indicating that there are different degrees of foreignness of the transplanted proteins in animals as well as in man. In man, by transplanting within the same blood group it is probable that the average life of homeografts might be somewhat prolonged. But there are such great differences within a given group which cannot be detected by the usual hemolysin or agglutinin tests, that permanent value from homeotransplantation is at present hopeless and must continue to be until some means is discovered to overcome the foreign protein reaction to the grafted tissue.

Heterotransplantation of thyroid in mammals never succeeds.

d. Diet. Diet notably affects both the structure and chemistry (166), (167), (168), (169). Baumann (96) in 1896 and many others (161) noted in dogs that fresh meats caused hypertrophy of the thyroid, while sea fish (cod) increased the iodin store. Watson (162) also found that a meat diet caused hypertrophy and hyperplasia of the thyroid cells in rats. Marine and Lenhart (163) (164), (165) showed that liver, particularly pigs' liver, was the most potent of a great variety of meats in causing thyroid hyperplasia in dogs and cats and also this food was an important factor in the causation of goiter in brook trout. Recent work by McCarrison (170), confirmed by Mellanby (171) showed that fats were even more potent in producing thyroid hyperplasia. McCarrison's suggestion that his effect is in part dependent on an increased bacterial putrefaction in the intestine seems unlikely. As thyroid hyperplasia is secondary to the depletion of the iodin store, these facts indicate that diets rich in proteins and fat increase the rate of discharge of iodin. It seems probable that thyroid activity is more necessary for the oxidation of fats and of proteins than of carbohydrates. Carbohydrate diets do not cause thyroid hypertrophy, as has been shown by McCarrison. Inanition brings about involution of the thyroid, decrease in the size of the epithelial cells and increase in colloid (172), evidence of decreased functional activity.

INTERRELATIONS. We are only at the beginning of definite knowledge concerning its functional interrelations with other tissues. During the last decade this subject has become involved in a stupendous mass of ill-advised speculation, exploitation and fiction. Interrelations may be either inhibitory (antagonistic) or augmentory in nature. Sufficient facts are available to indicate that these correlations determine the thyroid's most important effects on nutrition. These effects are brought about by acceleration and inhibition of its functional activity

which in turn are caused by chemical factors, positive or negative, acting through the blood stream either directly on the gland cells or indirectly through nerve impulses. Further, we must recognize that inhibition and acceleration of tissue activity may be brought about by the presence of a specific hormone or by its absence and that apparent acceleration may be an actual loss of inhibition and vice versa.

Thyroid-Parasex glands. (*Suprarenal cortex, interstitial and luteal cells.*) The relation of the thyroid to the sex glands was known to the ancients in its crudest external manifestations—the thyroid enlargement with menstruation, puberty, pregnancy and menopause (173). This relationship has passed down to our time with no proved additions to our knowledge concerning it (174). Recently it has been demonstrated that when the suprarenal cortex in rabbits is sufficiently injured as by freezing or by partial removal, a marked chronic increase in heat production usually occurs (80 per cent in a series by Marine and Baumann) (175). This increase usually begins within three to six days after the suprarenal injury and may last from two weeks to several months. Heat production may be increased to 60 per cent or more above the normal. On the other hand if the thyroid gland is removed and the metabolism allowed to fall to the myxedema level prior to the injury to the suprarenal cortex, this increase in heat production does not occur (82). Scott (176) has confirmed these findings, using cats. Golyakowski (177) in 1899 in a brief preliminary report observed increased CO_2 output in dogs following ligation of the suprarenal vessels. There is some evidence that the increased heat production is associated with a loss of iodin from the thyroid and recently Black, Hupper and Rogers (178) have published evidence that feeding "suprarenal gland residue" to dogs increased the iodin store of the thyroid. This reaction with increased heat production appears then to be a suprarenal cortex thyroid interrelationship. Our present interpretation is that the suprarenal cortex exercises a regulatory or inhibitory control over thyroid activity and when this is withdrawn the thyroid automatically responds with increased function. It should be pointed out that there is evidence that the suprarenal cortex exercises an inhibitory control over other tissue functions as well—the thyroid suprarenal interrelation, therefore, is not an isolated one. The practical application of these observations may be of great importance; for example the enlargement of the thyroid at puberty, during menstruation, pregnancy and menopause may be thus partly explained. The effect of bacterial toxins in causing thyroid hyperplasia may be in part determined by a primary

injury to the suprarenal cortex. Other well-known facts involving obvious interrelations as, for example, the hypersusceptibility of certain individuals to adrenalin or the hypersusceptibility of certain individuals to desiccated thyroid and thyroxin, probably have as their basis this fundamental thyroid suprarenal cortex interrelationship. Exophthalmic goiter is in some way intimately involved in this interrelation and the popular conception that this disease is a primary thyroid disease must give way to a broader conception in which cortical exhaustion indirectly, and epinephrin stimulation directly, are in my opinion important primary factors in bringing about increased thyroid activity. Enlargement of the suprarenal cortex with an inrease in the epinephrin store has been observed following prolonged feeding with desiccated thyroid (179), (180), (181), (182), (183). During starvation, enlargement of the suprarenal cortex has been observed. Both of these phenomena may be interpreted as an attempt to suppress thyroid activity.

Finally, the normal involution of the suprarenal cortex in infants should be mentioned. This remarkable destruction of the reticular and fascicular zones of the cortex has been observed only in infants and begins during the second or third week of extra-uterine life (183a). The process is initiated as a hemorrhagic infiltration of the two inner zones and goes on to necrosis, destruction and absorption of these layers with collapse and folding of the glomerular zone on to the medulla. The duration of the stages of absorption and healing is indefinite. Some authors estimate it at two to three weeks and other at two to three months. The end result, however, of this destruction is a marked decrease in the volume of cortex so that a child one year old has a smaller total volume of cortex than at birth.

The physiological significance of this rapidly progressive partial destruction of the cortex is unknown. It is not accidential or traumatic. Its occurrence in accessory suprarenals as well suggests that it is a systemic purposeful reaction to meet the altered conditions incident to extra-uterine life. In the light of the relation of experimental injury of the suprarenal cortex in rabbits, dogs and cats to increased heat production, it is suggested that one of the effects of the cortical destruction in infants may be increased heat production through thyroid activation. All that can be said at present is that the cortical injury and increased heat production in infants begin at approximately the same time and parallel each other.

Thyroid-Chromophil tissue. Epinephrin injected intravenously causes a marked constriction of the thyroid vesssels. An interrelationship of

function was first postulated by Eppinger, Falta and Rudinger (184). They assumed that the chromophil system directly stimulated the thyroid. This conception received experimental support from the work of Asher and his pupils (186), who in 1910 showed that the blood pressure response in rabbits to a given dose of adrenalin was greater after stimulation of the thyroid nerves with intact thyroid than before such stimulation. This has been confirmed from several sources and especially by Cannon and his co-workers. The Goetsch epinephrin test in exophthalmic goiter is a clinical application of this reaction. Oswald has shown that a similar increase in the reaction to epinephrin may be obtained by injecting iodothyreoglobulin instead of stimulation of the thyroid nerves. The nature of this reaction is still in doubt (187), (188). Asher and Flack believe that the thyroid hormone increases the irritability of or sensitizes the tissues innervated by the sympathetic nervous system in some way so that it is more susceptible to stimulation by epinephrin.

Thyroid-Gonad interrelationship. Very little is known (185). That there is an important direct relation between some constituent of the gonads, especially in the female, and the thyroid is certain. The exceedingly complex nature of the sex gland has thus far been a perfect barrier to trustworthy experimental investigation. Total removal of the gonads usually leads to a slight depression of the thyroid function (189).

Thyroid-Hypophysis. Rogowitsch (190) and others (191) (192), (195), (196) have reported marked enlargements of the anterior lobe and especially the pars intermedia following thyroidectomy—as much as 400 per cent. They interpreted this enlargement as indicating that the pituitary could function vicariously for the thyroid. Subsequent work by Simpson and Hunter (193) and by the author has not confirmed this. In rabbits there is a slight hypertrophy of the anterior lobe, but this is rarely more than 15 to 20 per cent after five or six months. Many investigators have found traces of iodin in both the human and sheep hypophysis—others have failed to find it. Simpson and Hunter (194) showed conclusively that the sheep pituitary contained no iodin even in animals recently fed with this element. Livingston (197) on the other hand has published a few observations indicating that desiccated thyroid protects male thyroidectomized rabbits against pituitary hypertrophy. No pituitary hypertrophy was observed in thyroidectomized female rabbits even without thyroid feeding. Hewitt (198), however, reported that thyroid feeding in white rats caused pituitary

enlargement. The idea that the thyroid and pituitary were functionally related seems to have been suggested by Virchow and was based on the morphological resemblance of the colloid filled follicles of the para intermedia to the thyroid follicle. Acromegaly is usually associated with a slight increase in the size of the thyroid and with an increase in heat production rarely over 20 per cent. None of the facts thus far established suggest any direct functional relation between the hypophysis and the thyroid.

Thyroid-Thymus. There is no evidence of any important relation between these two organs despite a relatively large literature dealing with their functional interdependencies (199). There is no doubt that the thymus is usually enlarged or persistent in many conditions in which the thyroid is involved, for example, simple goiter, myxedema, Graves' disease. Asher and Ruchti (200) found no change in the respiratory exchange after thymectomy in rabbits whether performed before or after thyroidectomy. Gudernatsch thought thymus feeding inhibited to some extent the action of the thyroid feeding on tadpoles. Baumann (unpublished) has found that foods enriched by the addition of protein-free nucleic acids of any origin stimulate growth in tadpoles. That there is an important indirect relation between the thyroid and thymus through the sex glands and suprarenals is certain since each of these glands is closely associated functionally with the sex and parasex tissues, and both the thyroid and the thymus are usually affected in conditions involving the suprarenals or sex glands, as for example, Addison's disease, status lymphaticus, castration, Graves' disease, etc.

Thyroid-Spleen. Asher and his pupils (201) have recently revived the question of the thyroid-spleen interrelationship suggested by Tauber (202) in 1884. Asher found that splenectomized rats with intact thyroids were less resistant to reduced oxygen pressures than normal rats, or rats with combined thyroidectomy and splenectomy. Splenectomy has been found to slightly increase the respiratory exchange in rabbits but this also occurs when the thyroid is removed.

Thyroid-Pancreas and Liver. Falta thought the thyroid and pancreas were antagonistic (203). He stated that an epinephrin injection which in normal dogs caused a marked glycosuria does not produce glycosuria in thyroidectomized dogs. Similar observations were made by Grey and de Sautelle (204) on dogs and by Pick and Pineles on goats. Underhill (205), however, denies that thyroidectomized dogs in which great care has been exercised to preserve the two external parathyroids are less susceptible to adrenalin glycosuria. Thyroid feeding was found

to produce a marked decrease in the diastatic activity of the pancrease of white rats and this was often associated with enlargement of the pancreas (206). Clinicians have reported the frequent association of some of the symptoms of Graves' disease with acute pancreatitis (207). It has also been suggested that the lowered sugar tolerance and glycosuria of Graves' disease might involve a thyroid-pancreas interrelation. The increased alimentary tolerance for glucose in myxedema or after thyroidectomy is of doubtful significance. It may be due to decreased absorption from the intestine rather than to a direct thyroidectomy influence. What evidence there is seems to indicate that any thyroid-pancreas interrelationship is an indirect one and dependent on epinephrin sensitization. Whipple and Christman (208) have shown that thyroidectomy does not influence the excretion of phenoltetrachlorphthalein by the liver.

Thyroid-Parathyroids. No interrelation of function is known. The earlier affirmative statements were based on faulty methods and errors in interpretation.

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INTRACELLULAR DIGESTION

The Enzymes and Anti-Enzymes Concerned

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All of the protozoa and many somewhat higher animals obtain their food by intracellular digestion. Among the lower metazoa including sponges, coelenterates and some of the lowest forms of worms, such as turbellaria, cells lining the digestive cavity act as phagocytes, ingest solid particles of food and dissolve them usually within vacuoles in their cytoplasm. Among the coelenterates and turbellaria food undergoes preparatory changes within the enteric cavity but the part of enzymes poured out into the cavity is doubtful. Extracellular digestion by enzymes secreted into a digestive tract is first definitely established in the ascending animal scale in the higher worms, such as nematodes, earth worms, etc., and becomes the sole method for the digestion of food.[1]

A proteolyte enzyme has been extracted from the bodies of amoebae and from the phagocytic cells of coelenterates, such as the sea anemones; it digests protein in a weakly acid and in a weakly alkaline medium. When the amoeba or the digesting phagocytes of higher forms ingest particles of blue litmus they are quickly turned to red but after a time the vacuole surrounding the granule loses its free acid and the indicator shows a neutral or alkaline reaction and many other indicators exhibit similar reactions.

In the lowest metazoa, namely, in the sponges, and in all higher forms which he examined, Metschnikoff (67) found mesoblastic phagocytes capable of approaching and ingesting foreign material which has found its way into the tissues of the organism. Among mammals the cells which exhibit these properties are notably the polynuclear leucocytes of the bone marrow and blood and certain mononuclear wandering cells which are widely scattered in the tissues. These cells approach

[1] The literature of phagocytic digestion of food in lower animals has been reviewed by O. von Fürth, *Vergleichende chemische Physiologie der niederen Tiere*, Jena, 1903 and by H. Jordan, *Vergleichende Physiologie wirbelloser Tiere*, Jena, 1913.

or fix foreign particulate matter which has entered the body, engulf it and so far as they are capable bring about the solution of the ingested material. Not only is foreign material subject to the activities of these cells but body substance, including cells and intercellular substance, which has been injured or killed, may undergo some change as the result of which it becomes the prey of the phagocytic cells and is eliminated by intracellular digestion.

When phagocytic cells of the higher animals are allowed to ingest indicators, such as litmus, tournesol-blue or alazarin sulphate, no change occurs. With neutral-red, bacteria and the nuclei of cellular elements ingested both by polynuclear leucocytes and by macrophages assume a brownish red color which Metschnikoff has attributed to a feebly acid reaction.

ENZYME OF THE POLYNUCLEAR LEUCOCYTES; LEUCOPROTEASE. The presence of peptone in pus was observed by Eichwald (16) in 1864, and later this observation was confirmed by Maixner (66) and by Hofmeister (31). In association with diseases characterized by pus formation, namely, empyema, purulent peritonitis, cerebro-spinal meningitis, etc., Maixner found peptone in the urine. The occurrence of proteolytic enzymes in the cells of purulent exudates was first demonstrated by Friedrich Müller (76), who showed that a glycerine extract of purulent sputum digests fibrin or coagulated protein in the presence of a weakly alkaline reaction. Similar enzyme was demonstrable in fresh pus from an acute abscess but was absent in the thin fluid from a "cold abscess." Leber (60) and later Achalme (1) showed that pus liquefied gelatin, and dissolved fibrin, egg albumin coagulated by heat, and casein.

Methods. Many methods used for the study of pepsin and trypsin are not applicable to the weaker enzymes present in phagocytic cells. Fresh fibrin frequently employed is not well adapted to the study because it contains enzymes which bring about autolysis. Some very simple methods have been introduced with the hope that they would be useful to physicians and numerous clinical studies have been made with their aid. The methods which have been most used will be cited briefly.

Liquefaction of gelatin: This method introduced by Fermi (21) has been used by Eppenstein (18) for the demonstration of enzymes in leucemic blood. Gelatin in the strength of 6 to 8 per cent is used as a substrate for the demonstration of enzymes. The substance to be examined is mixed in quantity of 0.2 to 0.5 cc. with 1 to 2 cc. of gelatin

containing 1 per cent soda and kept in an incubator at 37° to 40°C. during 12 or more hours. Should digestion occur the gelatin remains liquid after cooling with ice during several hours.

Digestion of casein: Gross (25) and Fuld (23) have introduced a method for the study of tryptic digestion which has been much employed in the study of the anti-enzymotic activity of the blood serum. Casein in 1 per cent solution in $\frac{1}{10}$ N sodium hydroxide is neutralized with $\frac{1}{10}$ N hydrochloric acid and diluted with five times its volume of physiological salt solution. After the enzyme has acted upon this substrate the mixture is acidified by 5 per cent acetic acid in weak alcohol and if digestion is complete the solution remains clear, since the digestion products of casein are not precipitated in acid solution, but if undigested casein remains it is precipitated. Numerous studies of the anti-enzymotic activity of the blood serum with carcinoma and other disease have been made by this method. The amount of serum necessary to inhibit a given quantity of enzyme is determined.

Serum plate method: Eduard Müller and Jochmann (71) have used, for demonstration of proteolytic enzymes, Loeffler's medium spread out as solid plates in Petri dishes. The medium consists of two parts of beef serum and one part of bouillon containing approximately 1 per cent of glucose and coagulated by exposure to a temperature from 85 to 95°C. during several hours. A drop of the material under investigation is placed upon the surface of the plate which is then incubated at from 55° to 60°C. during 24 hours. At this temperature a shallow cup-like depression is formed by the action of the enzyme upon the coagulated beef serum. Excavation of the surface of the plate does not occur at body temperature and the higher temperature has the advantage that it prevents the multiplication of bacteria. Various dilutions of the fluid to be tested for enzymotic activity may be prepared and the titer at which digestion ceases may be determined. Many drops may be applied to the same plate. This somewhat crude method has been widely employed for the study of enzymes and anti-enzymes both in health and disease but it has in great part served to confirm observations made by more accurate quantitative methods. The observations made upon leucocytic enzymes and anti-enzymes by this method have been fully reviewed by Weins (102) but he has overlooked almost all of the observations made by quantitative chemical methods.

Measurement of digestion by nitrogen determination: When the enzyme digests a protein substrate the activity of digestion is meas-

ured by the amount of protein coagulable by heat or by chemical means which has been split into incoagulable products such as albumose, peptone and lower nitrogen-containing decomposition products. This measurement is readily made by determining the amount of nitrogen in incoagulable substances before and after digestion during 24 hours at 37°C., some conveniently obtainable protein such as blood serum denaturalized by heat being used as a substrate. Nitrogen may be determined by the Kjeldahl method which was used by Ascoli and Maresche (4) and by Opie (79), or by the microchemical method of Folin, which Jobling (49) used. The method ensures an accurate determination of the activity of digestion.

Preparation and character of leucoprotease. The enzyme of the polynuclear leucocytes was extracted (Friedrich Müller) from purulent sputum or fresh pus by means of glycerine. A permanent preparation of the enzyme may be obtained from the leucocytes of a sterile inflammatory exudate produced by the injection of aleuronat into the pleural cavity of a dog by treating the washed cells with absolute alcohol in sufficient quantity to cause dehydration (Opie). After removal of the alcohol the cells are treated with ether, dried and reduced to a fine powder. This material actively digests protein such as blood serum denaturalized after dilution with an equal volume of physiological salt solution by heating to 75°C. It acts in a neutral or alkaline (0.2 per cent sodium carbonate) solution but is almost wholly inactive in the presence of acid (0.2 per cent acetic acid). This enzyme is slightly increased in activity by short exposure to temperatures between 50 and 60°C., is slightly impaired by a temperature of 65°C. and is destroyed between 70 and 75°C.

Fresh leucocytes incubated on Loeffler's serum plates at 37°C. cause no cupping of the surface but if leucocytes are first subjected to an elevated temperature (55°C.) and then incubated at body temperature active solution of the plate occurs. The high temperature according to Müller and Jochmann is required to destroy the leucocytes so that their enzymes may be set free. The enzyme causes active proteolysis at temperature between 50° and 55°C.

The proteolytic enzyme of the polynuclear leucocytes has been given the name "leucoprotease" by Opie (81).

A purified enzyme was prepared from leucocytes by Jochmann and Lockemann (57). Material containing the enzyme was allowed to autolyze during from 24 to 48 hours at 55°C. The autolysate was then treated with about five times its volume of alcohol (2 parts) and ether

(1 part) in order to remove fatty material and to precipitate protein. After standing during one day, the material was filtered and the residue, first evaporated to get rid of alcohol and ether, was intimately rubbed with glycerine in the proportion of about one-fourth of the volume of the original material from which the enzyme was obtained, and an equal volume of water was added. After standing one or two days in the dark the solution was passed through a porcelain filter and the clear filtrate was treated with five or six times its volume of a mixture of alcohol and ether. The white precipitate thus formed was dried in a vacuum over concentrated sulphuric acid. The final product which was yellowish brown and somewhat hydroscopic formed a brown solution when dissolved by rubbing with water or physiological salt solution. It digests coagulated blood serum, fibrin, gelatin and casein best in a weakly alkaline solution. When dissolved in water it is destroyed by temperatures between 70° and 75°C. but in dry form though impaired in activity resists temperatures from 75° to 95°C. being destroyed by 100°C.

The effects of various chemicals upon the enzyme of leucocytes have been studied by several observers, including Jochmann and Lockemann (57), Müller and Kolaczek (75). The enzyme is especially resistant to the action of formalin. Jochmann and Ziegler (58) noted that the proteolytic enzyme of leucemic organs was not destroyed by preservation in 10 per cent formalin even after the lapse of a year. If part of a spleen is placed in salt solution and part in 10 per cent formalin (Jochmann and Lockemann) disintegration proceeds rapidly in the former at a temperature of 55°C. so that complete liquefaction has occurred after 48 hours, but in the presence of formalin the tissue is completely preserved. If tissue preserved in formalin is washed in running water and then applied to the serum plate, excavation of the surface occurs. The experiment shows that digestion is inhibited by the presence of formalin but the enzyme is not destroyed. The purified enzyme of Jochmann and Lockemann was impaired but not destroyed when dissolved in 10 per cent formalin. It is noteworthy that the action of formalin on trypsin is similar to that upon the enzyme of leucocytes.

The production of fever by leucoprotease was observed by Jochmann (54). He found elevation of temperature in rabbits following the injection of enzyme of leucocytes or of pancreatin into the vein or into the subcutaneous tissue; it appeared after one hour, the temperature remained somewhat elevated after two hours and then fell. The result

was the same when the solution was heated during a quarter of an hour at 80° to 90°C. and is not dependent upon the presence of the proteolytic enzyme but is caused by substances related to proteins and associated with the enzyme. Jochmann thinks that these substances, as well as the toxins of bacteria, are concerned in the production of the fever which accompanies suppurative processes.

Diminution of the coagulability of drawn blood by addition of enzyme of leucocytes has been observed by Jochmann (54). Very large quantities of the enzyme injected into an animal cause an initial diminution of coagulability and later an acceleration of coagulation.

Occurrence of leucoprotease in the tissues. The proteolytic enzyme of polynuclear leucocytes which like trypsin digests in the presence of an alkaline reaction has been found in those organs within which polynuclear leucocytes are particularly numerous. Leucoprotease was found in the bone marrow by Opie (80). Autolysis of liver, kidney, spleen, lymph nodes and other tissues proceeds more rapidly in the presence of weak acid (e.g., 0.2 per cent acetic acid) than in a neutral or alkaline medium. Bone marrow, on the contrary, autolyzes more actively in an alkaline (e.g., 0.2 per cent sodium carbonate) than in an acid medium and furthermore digests extraneous protein under the same conditions. E. Müller and Jochmann (71) found that a drop of the material which was pressed from the cut surface of a human lymph node failed to digest the surface of coagulated serum incubated at 50°C. but under the same conditions active digestion was caused by red marrow squeezed out of a rib or vertebra.

Leucoprotease is formed within the polynuclear leucocytes before they leave the bone marrow. Here they elaborate a proteolytic enzyme which in some respects resembles trypsin. Nothing is known concerning the formation of the enzyme with the leucocyte. A zymogen has not been found. No relation to the specific granules of the polynuclear leucocytes has been demonstrated.

Hedin and Rowland (28) found that the expressed juice of the spleen of beef, horse, pig and sheep undergoes much more active autolysis in an acid than in an alkaline medium. Nevertheless in the presence of an alkaline reaction (0.2 to 0.37 per cent sodium bicarbonate) digestion is still considerable. Hedin (26) succeeded in separating almost completely two enzymes one of which, designated lieno-B-protease, digests in an alkaline medium. These enzymes not only digest the cells of the spleen but cause the disintegration of other proteins such as fibrin, casein and coagulated blood serum.

By quantitative determinations by means of the Kjeldahl method, Opie (80) found that the digestive activity of spleen in the presence of an alkaline medium stood next to that of bone marrow and exceeded that of liver, kidney and lymphatic nodes.

The proteolytic enzyme of leucocytes has been found by Friederich Müller and subsequent investigators in purulent exudates from patients. The studies of Opie were in great part made with the active leucoprotease obtained from sterile inflammatory exudates of dogs. E. Müller and Jochmann (72) using the serum plate method, have found no solution of the serum with cells obtained from rabbits and guinea pigs by injecting a solution of nucleinic acid below the skin or into the peritoneal cavity. Using the same method, Jochmann and K. Ziegler (58) have observed proteolysis caused by spleen and bone marrow of monkeys and slight proteolysis caused by the same tissues from the dog but have found none when they tested organs from fox, cat, various rodents, pig, sheep, beef and horse. The serum plate method is not sufficiently delicate to determine the absence of enzyme. A weak proteolytic enzyme digesting in the presence of an alkaline medium was found by Opie and Barker (84) in leucocytes obtained by injecting turpentine into the subcutaneous tissue of a rabbit; digestion was measured by Kjeldahl determination of nitrogen in the products of digestion. Using Van Slyke's micro-method to measure amino nitrogen, Parker and Franke (87) found only a very small increase after digestion of purified blood albumin and came to the conclusion that leucocytes of rabbits contain no proteolytic enzyme; they found, however, erepsin, which formed amino acids by digestion of peptone. No enzyme resembling the leucoprotease of mammals was found by Opie and Barker (84) in the leucocytes, bone marrow or spleen of the hen.

Relation of leucoprotease to trypsin. Leucoprotease which acts in the presence of an alkaline medium resembles trypsin but extracts obtained from leucocytes are much less active than preparations of trypsin. Those who have assumed that the anti-enzyme of the serum is a true antibody have reached the conclusion that leucoprotease and trypsin are identical for as Jochmann and Kantorowicz (56) have found, injection of animals with one of the two substances increases the inhibiting action of the serum for both. Furthermore, serum of which the inhibiting action is overcome by addition of one enzyme no longer inhibits the other. This evidence is inconclusive if it can be shown that the anti-enzymotic activity of the serum is caused by non-specific substances. This subject will by discussed under "Antileuco-

protease" and it will be evident that the more recent investigators find that the anti-enzyme of the serum is not a specific antibody.

Wiens and E. Müller (103) found that the blood serum of the turtle failed to inhibit the human leucocytic enzyme but inhibited trypsin as effectively as human serum. Jochmann and Kantorowicz (55) found that turtle serum inhibited very slightly the purified leucocytic enzyme whereas it actively inhibited trypsin in from 5 to 10 per cent solution, but human serum also inhibited trypsin more completely than leucocytic enzyme.

By the action of purified enzyme from leucocytes upon Witte's peptone in the presence of sodium carbonate (less than 1.5 per cent) at 37°C. under chloroform, Jochmann and and Lockemann (57) obtained crystals of trypsin after 5 or 6 days; parallel tests with pancreatin showed the presence of characteristic crystals after one day. Leucin, tryptophan and ammonia were demonstrable according to these observers as products of leucocytic digestion of peptone and they reach the conclusion that there is a very close similarity between the enzyme of leucocytes and trypsin.

A comparison has been made by Jobling and Strouse (49) between the products of digestion obtained from casein, purified egg albumin and Witte's peptone by the action of a solution prepared from dried powdered leucocytes on the one hand and of a solution made from the powdered trypsin of commerce on the other. The quantity of proteoses with leucoprotease exceeds that with trypsin whereas products of digestion below proteoses, namely, peptone and amino acids, were much less with leucoprotease than with trypsin. No tryptophan was formed by the action of leucoprotease. The digestion with leucoprotease in their experiments did not progress as far as tryptic digestion. They found in fresh pus cells an erepsin-like enzyme which was capable of splitting petpone.

Leucoprotease and immunity. Metchnikoff (68) proposed the name microcytase for complement or alexin which is concerned in bacteriolysis because he believed that it was derived from the polynuclear leucocytes and had the characters of a proteolytic enzyme. For complement which combines with antibody or amboceptor to cause hemolysis he suggested the name macrocytose believing that it was derived from macrophages. The effect of heat upon complement indicates that it is not identical with leucoprotease on the one hand nor with protease of macrophages on the other, since these substances resist temperatures which destroy complement.

Proteolytic enzyme of leucocytes was found to have no bactericidal action by Jochmann (53) when tested with B. typhosus, staphylococci and streptococci, nevertheless, according to Jochmann, it digested typhoid and colon bacilli just as quickly as it digested fibrin. Living bacteria resisted digestion and even multiplied actively in the solution of enzyme. The enzyme of leucocytes failed to cause hemolysis of red blood corpuscles. Jochmann found that the enzyme of leucocytes, unlike trypsin, failed to destroy diphtheria toxin.

The effect of leucoprotease upon pneumococci has been studied by Jobling and Strouse (50) and for control compared with bacterial autolysis in salt solution. After incubation during twenty-four hours the turbidity of the fluid containing leucoprotease is much diminished and within forty-eight hours the fluid is almost clear though the autolyzing suspension remains cloudy. In the presence of the enzyme pneumococci are reduced to shadows after twenty-four hours and are Gram-negative whereas the autolysing microörganisms are normal in appearance and still Gram-positive. Simple chemical reactions such as total acidity and formol titration indicate that bacteria are split into cleavage products which are lower than those obtained by autolysis. Formol titration as an index of the formation of amino-acids furnished evidence that leucoprotease causes proteolysis of pneumococci.

It is noteworthy that Jobling and Peterson (39), employing Folin's microchemical method for the determination of nitrogen have found no evidence of proteolysis when immune serum and complement cause bacteriolysis of typhoid and colon bacilli although proteolysis was demonstrable when trypsin acted upon the same microörganisms; they have not described parallel experiments with leucoprotease. The clearing of a bacterial suspension under the influence of immune serum does not prove that proteolysis has occurred, for Jobling and Strouse (52) found no parallel between the clearing of a suspension and the accumulation of products of protein disintegration.

Some of the effects of leucoprotease upon typhoid bacilli have been studied by Jobling and Bull (32). Typhoid bacilli like B. coli injected into the circulating blood of dogs causes elevation of temperature, bloody diarrhea, coma and death, and the abdominal organs, particularly the intestine, are intensely congested. This toxic substance is not destroyed by heat but is precipitated with the coagulable proteins of the bacteria so that the filtrate after heating to 100°C. is not toxic. If an emulsion of bacilli is subjected to the action of leucoprotease during from 2 to 5 days, the toxic element is no longer precipitated by

coagulation but is found in the filtrate obtained after heating to 100°C. The toxic substance is not specific and similar observations were made with B. coli, meningococcus and Staphylococcus aureus.

Observations of Kantorowicz (59) indicate that the resistance of living bacteria to proteolytic enzymes is referable, not to vital characters, but to anti-enzyme which is present in the bodies of the bacteria. Gram-negative bacteria lose this inhibiting property when heated to 70°C. but Gram-positive bacteria inhibit digestion even after boiling. Dried organisms retain their anti-enzymotic activity but lose their resistance when extracted with acetone. Bacteria were used in the foregoing experiments of Kantorowicz as substrate for proteolytic enzymes and digestion was measured by clearing of the suspension. Jobling and Peterson (39) measured digestion by Folin's method of nitrogen determination. They found that dried organisms resisted digestion in a degree proportional to their content of unsaturated lipoids (see anti-enzyme for leucoprotease). Substances which extract lipoids reduce the resistance of bacteria to proteolysis and the saponified extracted lipoids inhibit the digestion of casein by trypsin in a degree proportional to their unsaturation.

Bacteria, namely typhoid and colon bacilli, were found by Jobling and Peterson (39) to be so altered by immune serum and complement that they are more readily digested by trypsin. Complement alone or an excess of immune serum and complement rendered bacteria more rather than less resistant to proteolysis. Rosenow (91) noted that the intracellular digestion of pneumococci ingested by leucocytes *in vitro* varied considerably when the blood of different individuals was employed and came to the conclusion that the variation was referable to the action of the serum on the leucocytes stimulating them to increased activity. Douglas (15) treated B. pestis which undergoes phagocytosis in the absence of serum with unheated and with heated serum and subjected them to phagocytosis. Bacilli which had been treated with fresh serum underwent active intracellular digestion whereas those treated with heated serum remained intact within the substance of the leucocyte. Douglas further found that bacteria (bacillus of Friedländer) or red blood corpuscles exposed to the action of fresh serum and then washed by centrifugalization were digested by trypsin or by leucoprotease whereas bacteria or red corpuscles treated with serum heated to 60°C. remained unchanged.

INHIBITION OF LEUCOPROTEASE BY BLOOD SERUM; ANTILEUCOPROTEASE. To describe the enzymotic activities of phagocytic cells it is necessary to discuss the inhibitory factors which are concerned. The anti-enzyme of the blood serum will be discussed in relation to leucoprotease and observations concerning inhibition of trypsin will be cited in so far as they help to explain the inhibition of leucocytic enzymes. The terms anti-enzyme, antileucoprotease, etc., are used as convenient descriptive words and do not imply that the substances which inhibit are specific antibodies. The antitryptic action of the blood serum was first observed by Hildebrandt (30).

The power of the blood serum to inhibit the proteolytic activity of leucoprotease was described by Opie (79). The anti-enzyme for leucoprotease passes from the blood into an inflammatory exudate and has an important part in the phenomena of inflammation (see p. 572). The addition of blood serum or serum of an inflammatory exudate to a mixture of leucoprotease and substrate, such as denaturalized serum, gelatin or fibrin, prevents or, in smaller quantity, retards proteolysis. A temperature of 75°C. maintained during one-half hour sufficed to destroy the anti-enzymotic activity of serum, which was not impaired by a temperature of 65°C. The anti-enzyme was more effective in an alkaline (0.2 per cent sodium carbonate) than in a neutral medium but its inhibitory action was lost in the presence of acid (0.2 per cent acetic acid).

Employing the serum plate method E. Müller and Jochmann (71) found that serum inhibited the enzyme of leucocytes. E. Müller and Kolaczek (75) found no anti-enzyme in the cerebrospinal fluid; none was found in the bile nor in milk. In normal urine they found no anti-enyzme but with abundant excretion of albumin especially with chronic passive congestion of the kidneys and with chronic parenchymatous nephritis the urine inhibited the enzyme of leucocytes.

The anti-enzyme of the blood is not specific for leucoprotease obtained from the same species. Opie and Barker (84) found that leucoprotease of the dog is inhibited by serum of man, ox, dog, cat, goat and rabbit, the last mentioned being stronger than the others. Leucoprotease of rabbit was inhibited by the serum of dog and of rabbit, that of the rabbit being again the stronger. The serum of birds (pigeon, hen) on the contrary failed to exhibit anti-enzymotic action when tested with the leucoprotease of dogs. Wiens and E. Müller (103) found that the serum of the turtle failed to inhibit the proteolytic enzyme of leucocytes but inhibits trypsin almost as effectively as human serum.

Antileucoprotease was precipitated by Opie and Barker (84) with the albumin fraction of the blood serum, obtained by complete saturation with ammonium sulphate but was absent in the euglobulin fraction obtained by one-third saturation or in the globulin fraction obtained by half saturation.

By adding increasing quantities of leucoprotease to the same quantity of serum Opie (82) found that a given quantity of serum can inhibit the action of only a limited quantity of enzyme; for example, 2.5 cc. of serum completely inhibited the proteolytic activity of 20 mgm. of dried leucocytes but failed to prevent proteolysis when a larger quantity of enzyme was employed.

Anti-enzyme in the blood serum with disease. Numerous studies of the anti-enzymotic activity of the blood have been made; from the standpoint of diagnosis they have disappointed early expectations. No exhaustive review of the extensive literature of this subject will be made. Observations of interest in relation to the enzymes of leucocytes will be cited briefly.

The anti-enzymotic activity of the blood was studied by Wiens (99), (100), (101) in a considerable number of diseases; he has used the serum plate method and has performed his tests with fresh pus. With general and local pyogenic infections he had found a diminution of anti-enzyme which he attributes to an increased destruction of leucocytes in the body. Wiens and Schlecht (104) have compared the anti-enzymotic strength of the serum with the number of leucocytes in the blood and with acute infectious diseases have usually found diminution of anti-enzyme during and immediately after an increase in the number of polynuclear leucocytes; later an increase of anti-enzyme may occur. Nevertheless variations in the anti-enzyme particularly in chronic disease may bear no relation to leucocytosis.

The anti-enzyme of the blood is not infrequently increased with conditions which are unaccompanied by leucocytosis. Brieger and Trebing (10), (11) found antitrypsin increased with carcinoma and sarcoma in 90 per cent of the cases examined, and later, observing the same change with some other diseases, for example, tuberculosis accompanied by wasting and cachexia designated this increase "the cachexia reaction." Brenner (9) found that the anti-enzymotic activity of the serum was usually increased with severe anemia and chlorosis but no definite relation to the number of red blood corpuscles nor to the hemoglobin content of the blood was evident. Increase of antitrypsin was found by K. Myer (69) in association with exophthalmic goiter. Nevertheless,

increase of antitrypsin is often unassociated with wasting and anemia. Gräfenberg (24) and later Thaler (98) and others found increase of anti-enzyme during pregnancy.

Sir A. E. Wright (106) has found coincident loss of antitrypsin and diminution of alkalinity in the edematous fluid at the site of infection with the gas bacillus of Welch both in man and in animals. In the blood diminution of alkalinity and increase of antitrypsin was found.

Following the administration of potassium iodide, Jobling and Petersen (41) found diminution of the antitryptic content of the blood serum, but in two instances of iodide poisoning antitrypsin was increased.

The nature of the anti-enzyme in blood serum. Increase of anti-enzymotic activity of the blood was found by Jochmann and Kantorowicz (56) when they repeatedly injected subcutaneously into rabbits an extract of dried leucocytes from human pus. Before injection one drop of serum inhibited the proteolysis of one drop of pus but after these injections serum diluted from thirty-two to sixty-four times had the same effect. There was a parallel increase of anti-enzyme both for trypsin and for leucocytic enzyme. If trypsin was mixed with a quantity of serum which just served to inhibit its activity the mixture exerted no inhibitory action upon the enzyme of leucocytes. Upon the evidence of these experiments the authors reach the conclusion that the anti-enzyme for trypsin and that for the enzyme of leucocytes are identical.

Achalme (2) injected trypsin into the peritoneal cavity of guinea pigs and found the normal inhibitory action of the serum increased. After two months Bergmann and Bamberg (7) found the anti-enzymotic activity of the serum of dogs doubled by repeated subcutaneous injection of trypsin. The anti-enzymotic activity of the serum of two dogs which received 20 cc. of 4 per cent trypsin solution was increased after 24 hours in one instance to ten times its former strength. Some observers have assumed that the trypsin acts as an antigen and brings about the formation of a true antibody. Some have maintained that the anti-enzyme of normal serum is formed in response to the presence of enzyme within the body; Wiens (99) suggests that the enzyme of the leucocytes furnishes the stimulus to its formation. Colliner (12) thinks that trypsin absorbed from the pancreas, and K. Meyer (70) that autolytic and proteolytic enzymes derived from the tissues may act as antigen.

Specificity is one of the characters of antibodies formed when an antigen has entered the body. Eisner (17) found that sera which

varied in their anti-enzymotic action upon trypsin did not show corresponding variations when tested with labferment, pepsin, emulsin and lipase. Weil (105) cites evidence to show that anti-enzyme of the serum is not a specific antibody; human sera, he states, inhibit the vegetable protease papain in a constant ratio to their inhibition of trypsin. An observation of Pozerski (90) cited by Weil, is noteworthy in this connection; the serum of an animal immunized against papain contained a specific precipitin and an antibody which fixed complement but it possessed no increased ability to inhibit proteolysis caused by papain.

Increase of serum anti-enzyme following injection of trypsin is of little value as evidence of an immunity reaction, for similar increase is produced by a variety of apparently unrelated means. In animals which had received choloroform or phosphorus in quantity sufficient to produce profound intoxication Opie, Barker and Dochez (86) found an increase of proteolytic enzyme in the blood serum and in some instances complete disappearance of anti-enzyme. In animals which repeatedly received these substances in doses insufficient to cause death, there was a progressive increase of anti-enzyme. Braunstein and Kepnow (70) increased the antitrypsin of the blood by injecting an emulsion of tissue cells into the peritoneal cavity of rabbits. K. Meyer (70) found the antitrypsin of the blood increased in rabbits and dogs and also in patients after administration of thyroid tissue.

In view of the lack of evidence that the anti-enzyme of the serum is a true antibody attempts have been made to find in the serum some substance capable of inhibiting the action of proteolytic enzymes.

The antitryptic action of serum was attributed to lipoid substances by Schwarz (94). He found that an emulsion of lipoids prepared from the organs of the horse by extraction with alcohol and ether and precipitation by acetone inhibited trypsin. By extraction with ether serum was deprived of its inhibiting action but addition of lipoids restored it. He suggests that serum antitrypsin is a combination of lipoid with protein. Delezenne and Pozerski (14) had made the observation that serum treated with chloroform underwent autolysis. Sugimoto (96) found that the antitryptic action of egg white was removed by extraction with ether, petroleum ether, benzol and benzin, the effect of the last named being least. Addition of the lipoid which had been removed, of lecithin, of lipoid from brain and of lipoid from liver to the egg white extracted with petroleum ether did not restore its inhibiting action and the lipoids themselves had little effect upon the tryptic digestion of casein.

Leucoprotease and trypsin were inhibited by Jobling and Petersen (34) with sodium soaps prepared from olive oil, croton oil, cod liver oil and linseed oil. Complete inhibition of tryptic digestion of casein was caused by 0.005 gram of linseed oil soap and partial inhibition was evident with much smaller quantities. The anti-enzymotic activity of the soap was inversely related to the saturation of its fatty acid and in proportion to its iodine value. Soaps of the saturated fatty acids such as sodium stearate or palmitate did not inhibit the action of the enzymes. Saturation of an unsaturated fatty acid with iodine removed the inhibiting activity of the soap.

Studies of the nature of the serum antitrypsin have been made by Jobling and Petersen (37). The antitryptic action of serum, lost by standing under ether during 4 days was partially restored when, after evaporation of the ether, sodium hydrate was added in sufficient quantity to give the ether treated serum a slightly alkaline reaction. The anti-enzyme is removed when the serum is acidified with hydrochloric acid (e. g., 5 cc. N/10 acid added to 20 cc. of serum from the dog) and filtered through kaolin; extraction of the kaolin with sodium alcoholate furnished a solution which inhibited the action of trypsin and had about one-tenth the strength of the untreated serum. Potassium iodide was found to diminish the antitryptic action of serum. When linseed oil soap was mixed with serum its inhibiting action, like that of serum alone, was completely removed by heating during thirty minutes at 70°C. From the evidence of these experiments the authors reach the conclusion that the enzyme-inhibiting action of the serum is due to the presence of compounds of the unsaturated fatty acids.

Slovzov and Xenophontava (95) found that the antitryptic substance of the serum can be extracted with chloroform but not with toluol. Since fatty acids have the same antitryptic action which is reduced by iodizing they reach the conclusion that the antitryptic action of the serum is caused by lipoids.

The lipoidal nature of antitrypsin is not accepted by Cobliner (12), Meyer (70) nor Teale and Bach (97), all of whom have found that extraction of dried sera with lipoid solvents such as chloroform, ether and petroleum ether does not remove its antitryptic power. Teale and Bach cite experiments to show, contrary to the opinion of Jobling and Petersen, that lipoids are more readily extracted from the dry than from wet serum and maintain that the lipoidal solvents destroy the anti-enzymotic activity of the serum only when they bring about coagulation of protein. Their observations concerning the inhibiting properties of soaps are at variance with those of Jobling and Petersen and they reach the conclusion that the serum antitrypsin is protein in nature.

PROTEOLYTIC ENZYME OF MONONUCLEAR PHAGOCYTES (MACROPHAGES). All mammals and many lower vertebrate species possess two types of cells capable of engulfing and digesting solid particles. One type is the polynuclear leucocyte (polymorphonuclear) with fine granulations, the neutrophile leucocyte of human blood which with inflammation leaves the blood vessels and forms the chief cellular element of acute inflammatory exudates. Its enzyme, leucoprotease, has been described. The second type of phagocytic cell is larger than the polynuclear leucocyte, it has a large nucleus which is round, oval or somewhat irregular in outline but never lobulated like the nucleus of the polynuclear leucocyte, and its cytoplasm contains no granules exhibiting a specific reaction to dyes. These cells have been designated macrophages by Metchnikoff and are the endothelial leucocytes of Mallory. During the later stages of acute inflammation when the inflammatory irritant has been overcome, the number of mononuclear phagocytes increases and these cells are actively engaged engulfing and digesting within their substance polynuclear leucocytes, red blood corpuscles and other cellular elements. When recovery occurs they have an important part in removing cellular elements from the site of inflammation. Moreover, they ingest certain parasitic microörganisms such as the malarial parasites and other protozoa, the tubercle bacillus, B. leprae and some other bacteria. Their phagocytic activity in typhoid fever is well known.

The proteolytic enzyme found associated with these phagocytic cells by Opie (81), unlike the enzyme of the polynuclear leucocytes, digests with greatest activity in the presence of an acid medium. In the inflammatory exudate obtained by injecting aleuronat into the pleural cavity of a dog, the number of polynuclear leucocytes decreases with the progress of the inflammatory reaction whereas the number of mononuclear cells exhibits a corresponding increase. A comparison between the character of the exuded cells during the first five days of the inflammatory reaction and the proteolysis which they cause when completely freed from serum has shown that there is coincident with decrease of polynuclear leucocytes, decreasing capacity to digest in the presence of an alkaline reaction and coincident with increase of mononuclear cells increasing power to digest in the presence of acid. The lymph nodes adjacent to the site of inflammation, for example, with sterile pleurisy caused by aleuronat, the substernal lymph nodes, afforded an opportunity to study the enzyme of the large mononuclear cells almost wholly free from leucoprotease. In these nodes large

mononuclear phagocytes occur in increasing number during the first five days of the inflammatory reaction and during this period there is increasing ability to cause proteolysis in the presence of acid but no digestion in an alkaline medium. The lymph nodes which drain the inflamed tissue and are crowded with mononuclear phagocytes exhibit much greater ability to cause proteolysis than distant lymph nodes, such as those of the mesentery, which contain few phagocytic cells. The enzyme of the mononuclear phagocytes which accumulate as the result of an inflammatory reaction has been designated lymphoprotease by Opie. It causes active digestion of protein in the presence of weakly acid reaction (0.2 per cent acetic acid) but is almost entirely inactive in the presence of a neutral or alkaline reaction. The enzyme is more susceptible to heat than leucoprotease being much impaired in activity by temperatures between 60° and 70°C. It is destroyed by higher temperatures. It is destroyed by drying after treatment with alcohol and ether. It resembles very closely the autolytic enzyme of parenchymatous tissues.

Two proteolytic enzymes have been found in experimental inflammatory exudates in the dog by Jobling and Strouse (49). One acts in an alkaline medium and is isolated by drying with alcohol and ether. The other acts in an acid medium and, unlike the alkaline-acting enzyme, is destroyed by heating to 70°C. during one-half hour. In hyperplastic lymph node in which the sinuses contained many large mononuclear cells, Longcope and Donhauser (61) found an enzyme which digested protein in the presence of weak acid.

The studies of Lord (62) and Nye (78) upon the hydrogen ion concentration favorable to the enzymes of a pneumonic exudate will be cited.

Erepsin in leucocytes. The occurrence within leucocytes of an enzyme with the character of the erepsin of Cohnheim is indicated by observations of Jobling and Strouse (49). Leucoprotease obtained after drying leucocytes in alcohol and ether failed to decompose proteoses and peptone to form amino-acids and no tryptophan was demonstrable. Fresh leucocytes split proteoses and peptone into these products in both acid and alkaline media but heat at 70°C. during one-half hour destroys this property. The leucocytes apparently contain an erepsin-like enzyme which acts upon products of the digestion caused by proteolytic enzymes. Parker and Franke (87) found that extracts of leucocytes of rabbit failed to cause any significant disintegration of protein but digested peptone to form amino-acid which was measured

by van Slyke's method. Similar digestion of a suspension of typhoid bacilli was attributed to the disintegration of "bacto-peptone." They believe that these leucocytes of rabbits contain erepsin but no protease.

Petersen and Short (89) found an increase of peptone-splitting enzyme in the blood serum immediately preceding and accompanying the crisis of lobar pneumonia.

SERUM PROTEASE. Enzymes similar in their action to those of the leucocytes may be found in the blood serum and after the coagulation of the blood enzymes are found attached to the fibrin which is formed.

When blood serum was treated with chloroform by Delezenne and Pozerski (90) the serum caused proteolysis of gelatin and casein. In the blood serum of the ox Hedin (27) found a weak proteolytic enzyme which acts in the presence of an alkaline medium and is present mainly in the globulin fraction of the serum. By treatment of blood serum with weak acid (0.2 per cent acetic acid), Opie and Barker (85) demonstrated the presence of a proteolytic enzyme; the globulin fraction of the serum obtained by half saturation with ammonium sulphate contained an enzyme which digested protein in neutral or alkaline media but failed to act in the presence of acid. This alkaline-acting enzyme resembles leucoprotease which does not digest in the presence of acid. It is probable that the serum contains two proteolytic enzymes.

With chloroform poisoning in dogs of such intensity that the liver undergoes necrosis and the coagulability of the blood is diminished, Opie, Barker and Dochez (86) found that the blood serum acquires increased ability to digest protein; this increased proteolysis is referable to an enzyme which digests with maximum activity in a weakly acid medium. The enzyme which digests in the presence of an alkaline medium is not increased.

Autolysis of serum after addition of chloroform has been used by Jobling and his co-workers in a series of studies on enzyme action (42) as a measure of serum protease. This method does not serve to identify the protease with either of those which have been recognized in the serum. It is noteworthy that antitrypsin which they have simultaneously measured restrains enzymes like trypsin and leucoprotease which act in an alkaline medium but is inactive in the presence of the acid reaction which is favorable to the acid-acting protease of the serum. Protease was present in serum (33) in greatest strength in guinea pigs and rabbits and was found in cat, ox and dog. In the normal dog it was found occasionally but was constantly found with distemper, pneumonia and inanition. In normal human serum they found little or

no protease. Serum protease cannot be identified with complement for it resists chloroform which destroys complement and is not destroyed by a temperature of 56°C.

Jobling and Petersen (38) found that the serum of guinea pig, rabbit and horse loses its antitryptic action when treated with kaolin, starch, agar or bacteria. They refer the toxic symptoms produced by sera so treated (anaphylatoxin) to toxic split products formed by the action of serum protease upon the proteins of the serum itself.

Several observers claim to have demonstrated in sensitized animals the presence of enzymes specific for the protein used as antigen. Jobling, Petersen and Eggstein (44) found the serum protease practically unaltered after the first injection of foreign protein; with acute anaphylactic shock, on the contrary, there was an "instantaneous mobilization" of non-specific protease and decrease in anti-enzyme. Intoxication they think is the result of cleavage products (peptones) liberated by enzyme action immediately after the introduction of the antigen. They do not regard the almost instantaneous occurrence of shock as a valid argument against this view.

The Abderhalden reaction demonstrated the presence in the serum of a proteolytic enzyme which Abderhalden has maintained has a specific action on placental tissue and is present in the serum only during pregnancy. This opinion has been much disputed and several of those who have investigated the subject have reached the conclusion that the serum itself is the source of the dialyzable products which are obtained, the placental tissue being undigested. Jobling, Eggstein and Petersen (33) maintain that protease of the serum becomes active because under the conditions of the method, the restraining influence of the anti-enzyme is removed. A full discussion of this subject is not desirable in this review.

In a series of papers Jobling, Petersen and Eggstein (43), (46), (47), (48), have described changes in serum enzymes and anti-enzymes following the injection of trypsin, kaolin, proteoses, peptones and bacteria into dogs. There was an increase of protease and usually an increase of antitrypsin which they regard as lipoid in nature. The amount of demonstrable enzyme bore no relation to variations in temperature or in count of leucocytes. They believe that a disturbance of ferment-antiferment balance in the serum may result in proteolysis of serum protein with liberation of toxic split products but they admit that these are not the sole agents of bacterial intoxication.

In the absence of profound fatal intoxication such as that caused by chloroform or phosphorus, anti-enzyme is present in the serum in sufficient quantity to restrain the action of serum protease. A complete absence of anti-enzyme, Jobling, Petersen and Eggstein state, is not essential for protease action provided some absorbing surface is present on which the relative balance of ferment-antiferment may be altered; "it seems probable that the protease action can take place in what might be termed local areas of antiferment deficiency, such as must occur at the point of contact of the serum and absorbing surface." Petersen (88) has discussed the speculative basis for recent attempts to find therapeutic use for agents which modify the enzyme and anti-enzyme of the serum.

No evidence that neutral fats, fatty acids or lipoid bodies have a part in restraining the activity of serum protease has been found by Yamakawa (107). He measured proteolysis of serum by determining with the ninhydrin reaction the amount of dialyzable products formed from serum kept in a dialyzing thimble during 16 hours at 37°C. Serum of guinea pig undergoes proteolysis when treated with chloroform, methyl, ethyl and isobutyl alcohol and acetone but after removal of the chemical activator by vacuum, dialysis or extraction with indifferent chemicals (ether or petroleum ether) the original nonautolytic state does not return. Ethyl ether, petroleum ether, benzine and toluene neither activated nor paralyzed the serum protease. Addition of cholesterol, lecithin and neutral fats such as triolein and tripalmitin failed to influence autolysis.

FIBRIN-PROTEASE. Fibrin undergoes solution when allowed to stand in weakly acid, neutral or alkaline solution. The influence of varying temperatures upon solution and the formation of products of protein disintegration have shown that enzymes are concerned in the process. Studies of Rulot (92) furnish evidence that digestion of fibrin is caused by leucocytes imprisoned in the meshes of the fibrin. Proteolysis was measured by Kjeldahl determination of the nitrogen contained in decomposition products incoagulable by heat. To obtain fibrin free from leucocytes two methods were used. Clotting of drawn blood was prevented by addition of sodium chloride and the blood was centrifuged. Subsequent dilution caused the deposition of fibrin. In the second method the blood was made incoagulable by intravenous injection of propeptone; after centrifugalization, fibrin formation was brought about by a current of carbon dioxide. Pure fibrin obtained by these methods, Rulot found, was almost insoluble in physiological salt solu-

tion but underwent proteolysis when the leucocytes obtained by centrifugalization were added to it. In fibrin from the blood Barker (5) found a proteolytic enzyme which caused autolysis of fibrin and digested a protein substrate in presence of acid neutral and alkaline media.

In the fibrin of inflammatory exudates Barker (6) showed the presence of two enzymes. Leucoprotease was abundant in exuded fibrin in the early stage of acute inflammation produced by injection of turpentine into the pleural cavity of a dog and could be separated from the second enzyme which acts in acid by drying fibrin after treatment with alcohol and ether. After the second day of inflammation leucoprotease had disappeared but a proteolytic enzyme acting in an acid or neutral medium was abundant.

INFLAMMATION. At the site of an inflammatory reaction anti-enzyme of the blood passes with the exudate into the inflamed tissue or cavity and the leucocytes are surrounded by a fluid capable of inhibiting the proteolytic enzyme which they contain. The polynuclear leucocytes engulf solid particles and digest them within vacuoles in their substance. The anti-enzyme of the surrounding fluid serves to limit the activity of the enzyme to the site in which it is effective. Should the leucocyte be destroyed the enzyme which is set free can no longer cause proteolysis. With relatively mild inflammation such as that which occurs with lobar pneumonia or with serofibrinous pleurisy there is no solution of tissue and the part is ultimately restored to normal.

Study of enzymes has served to explain many of the phenomena of resolution. At the height of the inflammatory reaction the solid elements of the exudate are polynuclear leucocytes, red blood corpuscles, in small number, other cellular elements and fibrin. Leucoprotease appears to be chiefly concerned with the digestion of protein particles, such as bacteria engulfed by the polynuclear leucocytes. The mononuclear phagocytes which accumulate during the later stage of an inflammatory reaction which is proceeding toward recovery digest within their substance polynuclear leucocytes, red blood corpuscles and other cells. This phenomenon occurs not only at the site of inflammation but in the sinuses of the regional lymphatic nodes to which cellular elements as well as fluid are carried by way of the lymphatics. The enzyme of the large mononuclear phagocyte resembles the autolytic enzymes widely distributed in the parenchymatous tissues and is doubtless capable of causing autolysis and final disappearance of the macrophage under suitable conditions. It is often assumed that autolysis of the exudate is caused by the leucoprotease of polynuclear leucocytes but the correctness of this view has not been established.

When experimental pleurisy is produced by intrapleural injection of a sterile inflammatory irritant such as turpentine, serum accumulates and fibrin is deposited on the pleural surfaces. Accumulation of fluid reaches a maximum after 3 or 4 days and subsequently fluid rapidly subsides so that it has disappeared after 6 or 7 days. Reactions showing the presense of peptone and albuminoses were obtained by Opie (83) on and after the third day. Leucoprotease was present in the fibrin which was removed from the cavity during the first few days of inflammation and perhaps at this period had a part in causing its solution but later at a time when fluid had disappeared from the cavity this enzyme was no longer demonstrable and the fibrin which in diminishing amount was present in the pleural cavity during the next five or more days underwent autolysis only in the presence of weak acid. Final disappearance of fibrin is evidently brought about by an enzyme which has the characters of lympho-protease and resembles the autolytic enzymes of the tissues. It is difficult to determine what are the factors which bring this enzyme into action. It is noteworthy that the alkalinity of the exudate was less than that of the blood and diminished slightly with the progress of the inflammatory reaction.

When an inflammatory reaction is of such intensity that leucocytes accumulate in immense numbers and solution of tissue and of fibrin occurs, suppuration is established and resolution with restoration to normal is no longer possible. Opie (82) found that disintegration of polynuclear leucocytes in a purulent exudate sets free leucoprotease in quantity sufficient to completely overcome the anti-enzyme of the exuded serum. Both the whole pus and its fluid part separated by centrifugalization from the pus cells was now capable of causing proteolysis *in vitro*. The proteolytic activity of the exudate unrestrained by anti-enzyme explains the solvent action of pus for injured tissue and fibrin. An inflammatory irritant such as turpentine injected into the pleural cavity of the dog, where fluid rich in anti-enzyme readily accumulates, causes a serofibrinous inflammation which undergoes resolution with restoration of the cavity to normal. An equal quantity of the same irritant injected into the subcutaneous tissue where fluid accumulates with difficulty causes on the contrary very extensive suppuration with solution of tissue and healing by scar-formation.

In pus produced by pyogenic cocci, for example, with acute peritonitis, E. Müller and Kolaczek (75) found that the anti-enzyme was "saturated" by enzyme derived from leucocytes which had undergone disintegration; the fluid overlying the cells after centrifugalization exhibited proteolytic activity.

Since the normal spinal fluid, unlike other body fluids, contains neither enzyme nor anti-enzyme, Dochez (13) has studied the changes which occur with inflammation. With epidemic meningitis anti-enzyme may enter the spinal fluid but quickly leaves it. With the more severe inflammation caused by pneumococcus or streptococcus leucoprotease derived from disintegrating polynuclear leucocytes gives the fluid proteolytic activity which may itself act as an irritant and increase the severity of the disease.

In the exudates from acute arthritis, pleurisy, and wounds of war, E. Zunz (109) at La Panne in Belgium studied the relation of the anti-proteolytic property of inflammatory exudates to their alkaline reserve. The fluid was separated from the cells of the exudate and the quantity of this fluid necessary to inhibit a standard solution of trypsin was determined and designated the antitryptic index. The hydrogen ion concentration of the serum entirely freed from carbon dioxide was determined by the method of Marriott. The tryptic index and the alkaline reserve of serous or hemorrhagic exudates never exceeded and were almost always less than those of the blood serum of the same individual. The fluid from purulent exudates did not usually inhibit tryptic digestion and on the contrary digested coagulated egg white but occasionally this fluid had a slight antitryptic action. The alkaline reserve of seropurulent and purulent exudates was almost always less than that of serous exudates; increase of alkaline reserve was often parallel with an increase in the number of leucocytes and a diminution of the antitryptic index but the alkaline reserve tended to return to normal when the leucocytes diminished and the antitryptic index increased.

In serous exudates Zunz has found that diminution of complement is often parallel with diminution of antitryptic activity but this relation is not constant. In purulent exudates with complete or almost complete loss of antitryptic action complement has disappeared. Since the enzyme of leucocytes is present in excess the observation furnishes evidence, were more needed, to show that complement (microcytase) and proteolytic enzyme of leucocytes are not identical. Opie found that opsonin disappeared from an inflammatory exudate when it became purulent. It is probable that the loss of these bodies is referable to the action of unrestrained enzyme.

Pneumonia. The autolysis of the consolidated lung of lobar pneumonia was studied by Friedrich Müller (77); albumoses, leucin, tyrosin and other products of protein disintegration were formed at body temperature and nuclei disappeared as the result of decomposition of neu-

cleins. Flexner (22) found that autolysis took place much more quickly and perfectly in lungs in the stage of gray than of red hepatization and attributed the difference to the greater number of leucocytes with gray hepatization. Autolysis of the lung in instances of unresolved pneumonia was slow and incomplete.

By means of the serum plate method Lord and Nye (64) have studied the relation of enzyme to anti-enzyme at different periods of the disease. Sputum and exudate obtained at autopsy in the later stages of lobar pneumonia erode the serum plate. In the early stage of the disease proteolysis does not occur but if the cellular material obtained from the exudate is separated from the serum which accompanies it active digestion occurs. In this early period enzymes are inhibited by anti-enzymes. The author suggests that excessive impairment of circulation or excess of enzyme may result in disintegration of the pulmonary framework and abscess formation whereas excess of anti-enzyme may prevent resolution.

The hydrogen ion concentration favorable to the action of proteolytic enzymes contained in the cells derived from the pneumonic lung has been studied by Lord (62) and by Nye (78). They have used the serum plate, gelatin and peptone as substrate. The proteolytic enzymes are active with hydrogen ion concentrations between 7.3 and 6.7 and are inactive with higher, that is, more acid concentrations. Lord found an enzyme which splits peptone to form amino-acids; it acted with hydrogen ion concentrations between 8.0 and 4.8 but was most active between 6.3 and 5.2. He suggests that there is a gradual increase of the hydrogen ion concentration of the pneumonic exudate; the proteolytic enzyme or enzymes which act in a weakly alkaline and weakly acid medium are perhaps inhibited by the increasing hydrogen ion concentration but the peptone splitting enzyme is further activated when the hydrogen ion concentration of the exudate is increased to the range of its optimum activity.

In the blood serum of patients with lobar pneumonia Ascoli and Bezzola (3), using the casein method of Fuld and Gross, found an increase of antitryptic activity in the earliest stage of the disease; this increase is maintained for a time and is followed by a decrease which occurs after the crisis. They have suggested that these changes are referable to a kinase which is derived from leucocytes and activates pancreatic trypsin; upon the basis of this speculation increase of antitrypsin is regarded as a reaction which follows the liberation of kinase by disintegration of leucocytes in the pneumonic exudate. Using the serum plate method to

test the strength of serum anti-enzyme for enzyme of pus cells Bittorf (8) found decrease of anti-enzymotic activity during the crisis followed by a considerable increase immediately after crisis and subsequently return to normal.

A study of *a*, the antitrypsin of the serum, its effect upon tryptic digestion being measured by Folin's method for nitrogen determination; of *b*, serum protease, measured by autolysis of serum after treatment with chloroform; and of *c*, non-coagulable nitrogen-containing substances of the blood, has been made by Jobling, Petersen and Eggstein (45). They find that the crisis of pneumonia is usually accompanied by *1*, a decrease of the serum antiferment which before the crisis is increased; by *2*, a mobilization of non-specific protease in the serum; and by *3*, a decrease in the non-coagulable nitrogen and proteoses in the serum. They suggest that toxic split products of fibrin and of leucocytes rather than toxic products of the pneumococcus dominate the symptomatology of lobar pneumonia; crisis is perhaps the beginning of active autolysis depending upon an altered relation between ferment and antiferment so that with rapid autolysis toxic materials are destroyed.

In pneumonia that terminates by crisis or by lysis Petersen and Short (89) found that an increase of ereptase (peptidase, erepsin) invariably precedes or accompanies the change of clinical symptoms associated with crisis or lysis, but in instances in which death occurs the ereptase in the serum is usually less than that in the serum of a normal individual.

Leucemia. The presence of incoagulable albumose-like substances in leucemic blood removed from the body after death was recognized by E. Ludwig (65). Erben (20) found that peptone and albumoses were not present in the blood when it was drawn but appeared after plasma and leucocytes separated from red corpuscles had stood in the incubator under aseptic conditions during three days. When the precipitate formed by treating leucocytes and plasma with alcohol was dried and extracted with glycerine the extract digested fibrin but lost this property when boiled. Normal blood and blood from cases of lymphatic leucemia did not undergo the change found with spleno-myelogenous leucemia. In cases of myelogenous leucemia, Schumm (93) demonstrated that products of protein disintegration were not present in the blood when it was removed from the body but appeared after the blood had been allowed to stand under chloroform. He attributes the change to a proteolytic enzyme.

E. Müller and Jochmann (71) showed that a serum plate was excavated by the blood of myelogenous leucemia when kept at 55°C. The blood of lymphatic leucemia did not produce these changes. Müller and Jochmann and Eppenstein (18) showed that the enzyme was in the cells of the blood and was inhibited by the serum.

Using the serum plate method, Jochmann and K. Ziegler (58) found that the bone marrow and spleen from cases of myeloid leucemia caused very active digestion; lymph nodes caused digestion in proportion to the myeloid transformation which they had undergone. Bone marrow of normal individuals caused active digestion of the serum plate, spleen caused slight digestion and lymph node none. Organs from cases of lymphatic leucemia and pseudo-leucemia, they state, cause no more digestion than those from normal individuals.

Studies have been made to determine if the large basophile cells of acute leucemia contain a proteolytic enzyme similar to that present in the myelocytes of myelogenous leucemia. In a case designated acute myeloid leucemia, K. Ziegler and Jochmann (108) found that half of the nucleated blood cells in the bone marrow and spleen were pure basophile cells or transitions between these and granular myelocytes; these tissues caused very active proteolysis of serum plates. Eppenstein (19) found no difference in enzymotic activity between the large lymphocytes of the blood from a case of acute lymphatic leucemia and the small lymphocytes from the chronic type of the disease. Müller and Jochmann (74) state that the blood cells in a case of acute lymphatic leucemia acted upon coagulated blood serum in the same manner as polynuclear leucocytes. Longcope and Donhauser (61) studied a case classified as acute lymphatic leucemia because the blood contained large mononuclear cells of the type of large lymphocytes with abundant faintly basophilic usually nongranular cytoplasm. Nevertheless a few neutraphilic myelocytes were found in the blood. The cells from the blood drawn during life and from lymphatic nodes obtained at autopsy caused proteolysis, measured by the Kjeldahl method, in an alkaline medium, and in this respect differed from the small lymphocytes of chronic lymphatic leucemia which caused no digestion and from the large mononuclear ("endothelial") cells of a hyperplastic lymph node which digested only in the presence of acid.

With myelogenous leucemia Jochmann and Kantorowicz (56) and Wiens (101) found no definite alteration of the anti-enzyme of the blood so that with a very high leucocytic count it may be normal. Wiens (102) suggests that enzyme set free by destruction of granular leucocytes

may stimulate formation of anti-enzyme so that the two are balanced. In fatal cases, he states, there may be a loss of anti-enzyme before death so that the serum may cause proteolysis.

Tuberculosis. Caseous material from tuberculous lesions or the lung with tuberculous pneumonia did not undergo the active autolysis which Friedrich Müller (77) observed with lobar pneumonia. Heile (29) thought that the failure of caseous material to undergo absorption was due to absence of enzyme rather than to insolubility of caseous material. He found that the contents of a "cold abscess" did not digest fibrin but after injection of iodoform in glycerine polynuclear leucocytes appeared and the exudate now dissolved fibrin. E. Müller and Jochmann (71) found that fluid from a "cold abscess" unlike pus of acute inflammation did not excavate the surface of serum plates incubated at 55° to 60°C.

The presence in tuberculous tissue of a proteolytic enzyme digesting in a weakly acid medium was demonstrated by Opie and Barker (85). This enzyme resembled that of the large mononuclear phagocytes and digests protein more actively than the similar enzyme of parenchymatous organs such as the liver. The presence of tubercles within the liver increases the proteolytic activity of the tissue in neutral or acid media. These observations are in accord with the well-known histological characters of the tubercle which in great part consists of mononuclear cells capable of acting as phagocytes. The enzyme of tuberculous tissue is most abundant at a time when caseation is beginning but with advance of caseation its activity diminishes and with complete caseation total disappearance of enzyme seems to occur. Leucoprotease digesting protein in the presence of an alkaline reaction was found only when the tuberculous tissue was first formed and, examined microscopically, was found to contain some polynuclear leucocytes.

No autolysis of caseous material from lymph nodes first dried and ground and then suspended in water was found by Jobling and Petersen (36) unless the tuberculous nodes had become secondarily infected. When the lymph nodes were infected, autolysis occurred in the presence of alkaline and of acid reaction but was much more active in the latter instance. Affected areas in lungs with caseous pnemonia were freed as much as possible from less involved lung tissues, dried, ground, suspended in water and subjected to autolysis. The authors assume that the caseous material was not completely freed from inflammatory exudate. Autolysis was active in the presence of acid but absent when the medium was alkaline. With the purpose of removing inhibiting substances from the material under examination Jobling and Petersen

first treated some of it with acidified alcohol, centrifugalized, washed with alcohol and ether and dried. It then underwent autolysis in the presence of an alkaline reaction. The authors do not discuss the origin of this enzyme which, like leucoprotease, acts in an alkaline medium, and do not appear to have examined the tuberculous lung tissue to determine if it was secondarily infected with pyogenic microörganisms or contained polynuclear leucocytes.

From the bodies of tubercle bacilli Jobling and Petersen (35) have obtained an extract which inhibits the action of trypsin and of leucoprotease. An extract of tubercle bacilli made with ether and alcohol did not inhibit and an extract was prepared as follows: the ether-alcohol extract was dissolved in ether, precipitated with acetone, evaporated to dryness and saponified with alcoholic potash; the resulting soap was dissolved in water and repeatedly extracted with petroleum ether, treated with hydrochloric acid to liberate fatty acid, taken up with ether, washed with water and resaponified. An antitryptic agent was obtained. Though the soap obtained had a lower iodine value than soaps previously prepared from linseed, olive and cod liver oils, it had a greater inhibiting activity. This inhibiting action was lost when the soap was saturated with iodine. The same observers have extracted from caseous material of tuberculous lymph nodes and of the lung with caseous pneumonia, inhibiting agents with similar characters. They believe that these observations explain the failure of caseous material to undergo disintegration through the action of phagocytes or by autolysis but they do not assume that the inhibiting agent is wholly derived from the tubercle bacillus for they suggest that autolysis of the necrotic material of an anemic infarct is prevented by similar factors.

It is noteworthy that the observations which show that soaps of unsaturated fatty acids are anti-enzymotic agents have reference to trypsin and to leucoprotease. There has been no suggestion that trypsin is present and it is well known that polynuclear leucocytes in the absence of secondary infection are an inconspicuous constituent of tuberculous tissue and are not usually found in or about a caseous focus or an anemic infarct. Jobling and Petersen (41) have assembled evidence to show that potassium iodide which in moderate doses, they find, diminishes the antitryptic activity of the blood serum, hastens the softening of caseous material. A similar explanation is offered to explain the absorption of syphilitic gumma caused by potassium iodide. Nevertheless there are no observations to show that enzymes such as leucoprotease which are inhibited by the anti-enzyme of the blood serum are concerned in the absorption of the tubercle or of the gumma.

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THE PHYSIOLOGY OF CREATINE AND CREATININE

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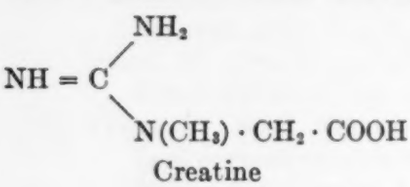
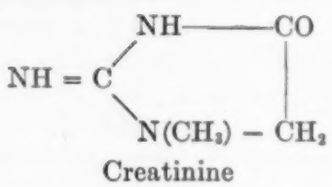
Creatine is α-methylguanidine-acetic acid, or methylglycocyamine, and may accordingly be looked upon as a derivative primarily of acetic acid, or of glycocoll, sarcosine (methylglycocoll), guanidine, methylguanidine, or glycocyamine (guanidine-acetic acid). From the standpoint of purely animal biochemistry one of its most distinguishing structural characteristics is its N-methyl radicle, which brings it into relation with the betaines and with choline. Its guanidine group, on the other hand, suggests a connection with arginine, the only known derivative of guanidine among the products of protein hydrolysis.

Creatinine, 2-imido-5-keto-3-methyl-tetrahydroimidazole, or methylglycocyamidine, is the internal anhydride of creatine, to which therefore it is related in the same way as glycocyamidine to glycocyamine. The ring resulting from the loss of a water molecule is built upon the imidazole (or glyoxaline) nucleus, so that creatinine possesses, besides the relations dependent upon its derivation from creatine, others with such biochemically important imidazole derivatives as histidine and the purines. Among the latter guanine, 2-amino-6-oxypurine, may claim a two-fold connection with creatinine, for in addition to the imidazole nucleus common to every purine its structure includes, as part of its pyrimidine complex, a guanidine residue.

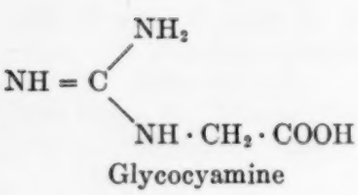
The structural interrelationships of the compounds mentioned are made obvious in the accompanying formulae:



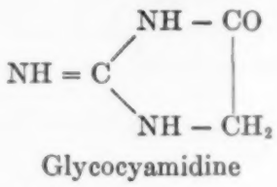
Creatine



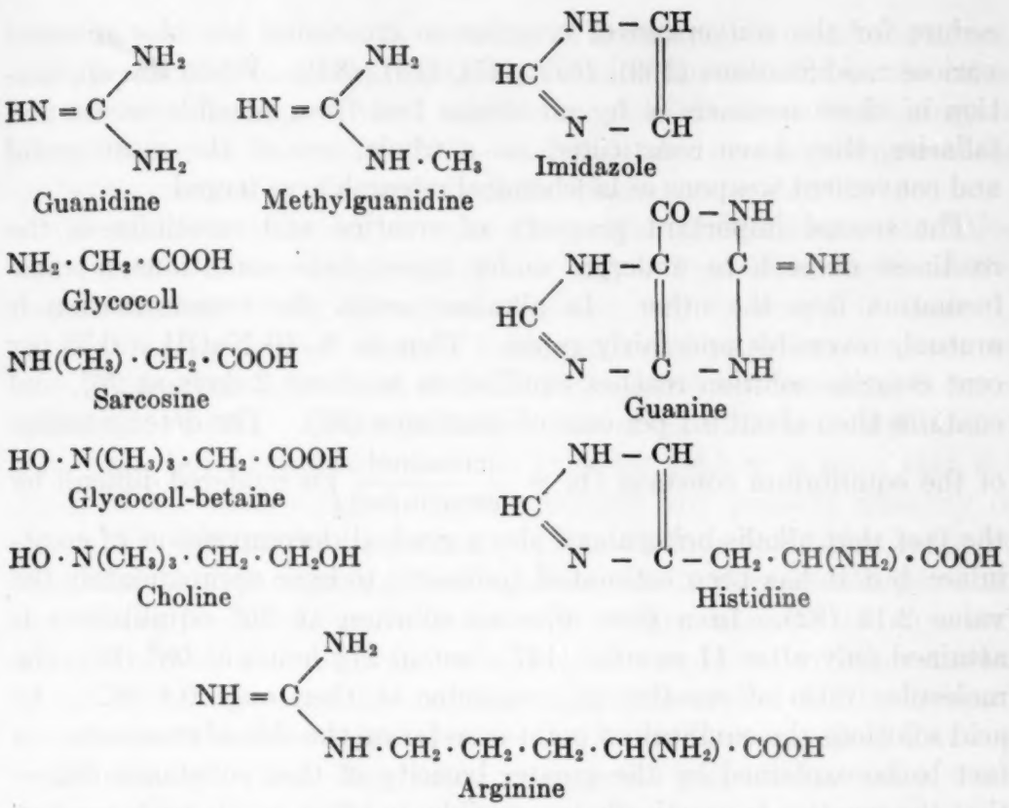
Creatinine



Glycocyamine



Glycocyamidine



Of the chemical properties of creatinine and creatine two are of special importance from the point of view of the biochemist.

The first is the power of creatinine to react with alkaline sodium picrate with the formation of an intensely red reduction product, probably sodium picramate. Upon this reaction, first described by Jaffé (105), Folin (61) based a colorimetric method for the determination of creatinine, from the introduction of which in 1904 all sure progress in the quantitative study of creatine-creatinine metabolism can be dated. Folin (61), (64) further utilized the reaction in a method for the indirect determination of *creatine*, involving its conversion into creatinine by heating under suitable conditions (see below) with an acid. In their original form these methods were adapted only to the determination of relatively considerable quantities of "total" or preformed creatinine, but they have been variously modified, by Folin himself and by others, so as to be applicable to the analysis of very small volumes or very great dilutions of urine (184), (67), or to the determination of creatine and creatinine in muscle (160), (184), (68), (12), (145), (107), (14), (15), blood (184), (68), (148), (214), (51), (79) (71), tissues generally (184), (68) and milk (68). The original pro-

cedure for the conversion of creatine to creatinine has also received various modifications (169), (67), (27), (13), (83). While the application of these methods is by no means free from possible errors and fallacies, they have constituted, as a whole, one of the most useful and convenient weapons of biochemical research ever forged.

The second important property of creatine and creatinine is the readiness of each to undergo, under appropriate conditions, a transformation into the other. In alkaline media the transformation is mutual, reversible and fairly rapid. Thus in N/10 NaOH a 0.38 per cent creatine solution reaches equilibrium in about 2 days at 26°, and contains then about 0.1 per cent of creatinine (82). The determination of the equilibrium constant $\left(K = \frac{[\text{creatine}]}{[\text{creatinine}]}\right)$ is rendered difficult by the fact that alkalis bring about also a gradual decomposition of creatinine; but it has been estimated indirectly to have approximately the value 2.12 (82). In a pure aqueous solution at 36° equilibrium is attained only after 11 months (147), but in 21½ hours at 98° (83); the molecular ratio of creatine to creatinine is then only 0.4 (82). In acid solutions the equilibrium point is so far on the side of creatinine—a fact to be explained by the greater basicity of that substance (82)—that the reaction is practically irreversible; in other words acids convert creatine completely into creatinine, but leave the latter unaffected. The velocity of the conversion, for any given concentration of creatine, depends on the temperature and on the concentration of hydrogen ions. In normal HCl the change, for small concentrations of creatine, is complete in 15 days at 26°, and within 24 hours at 60° (82), (83); in N/2 HCl it takes about 3 hours at 98° (64), and 15 minutes at 117° (25).

Biological distribution of creatine and creatinine. Creatine and creatinine are known with certainty only as products of the metabolism of vertebrates. Reports of the presence of creatinine in the culture media of bacteria (10), (75), (60), (178), (179), (137), or, along with creatine, in the tissues of *Abalone*, a mollusc (8), are based as yet only upon imperfectly specific color reactions.

Creatinine is a constant constituent of the urine of mammals, occupying as a rule a place among the organic nitrogenous catabolites second in quantitative importance only to that of urea. In the urine of birds it is relatively less abundant, being replaced there largely, although apparently not entirely, by creatine (156), (196). Reptiles appear in this respect to resemble birds (124). The meager data available regarding fishes (44), (45) seem to put elasmobranchs with the mammals,

teleosts with the birds. The position of the amphibia is unknown; van der Heyde (89) could find neither creatinine nor creatine in the urine of hibernating frogs. Most adult mammals—ruminants forming, it would seem, an exception (153)—excrete under normal circumstances either no creatine at all or only irregular traces.

Creatinine is believed to occur also, although in minute quantities only, in the tissues and fluids of the body. Typical examples of the concentrations which have been reported will be found in table 1. The data are derived entirely from micro-analytical applications of the Folin method, and are undoubtedly subject to considerable error (102), (214), (79). In the particular case of blood Behre and Benedict (20) have indeed recently produced evidence tending to show that the error of the determination greatly exceeds any possible quantity of creatinine that might be present. Their findings should probably be regarded as still requiring confirmation; but if they should prove to be correct for blood, grave doubt will be thrown upon the supposed concentration, and even the existence, of creatinine in other tissues also. For the present the creatinine figures of table 1 will be assumed to possess at least a relative value; but it must be recognized that any conclusion of which they form an essential premise lies under some suspicion.

Creatine is a characteristic component of certain special tissues, and is possibly, as indicated again by the quantitative data of table 1, not altogether lacking in any. Its presence has been unequivocally demonstrated, either by actual isolation in substance or by production, after dehydration, of the characteristic creatinine zinc chloride, in the cases of striated muscle of the vertebrates generally (including mammals, birds, amphibians, fishes, and the lamprey), of mammalian heart muscle, of brain (21), and of the blood as a whole (20). It has been shown, by the same criterion, that blood serum contains certainly either creatine or creatinine or both (121). For the other tissues named in table 1 (including, as far as I have been able to discover, the smooth muscles of vertebrates) the evidence for the occurrence of creatine is limited to the positive outcome of the Jaffé test after treatment of the tissue or its extract with acids. Where this test indicates really notable quantities of creatine, as with the testis, it is perhaps justifiable to assume that actual creatine could be obtained from the tissue if anyone took the trouble to seek it; but where its reported concentration is as low as it is with smooth muscle and with the majority of the non-muscular tissues, a certain amount of scepticism regarding its presence is excusable.

TABLE 1

Creatine and creatinine content of tissues

(Mgm. per 100 gm.)

TISSUE	ANIMAL	CREATINE	CREATININE	AUTHORITY
Striated muscle	Man (adult)	393–430	10.0–11.5	(184)
Striated muscle	Man (1¾ years)	331		(46)
Striated muscle	Man (at birth)	190		(168)
Striated muscle	Rabbit	494–540	3.4– 9.2	(140)
Striated muscle (white)	Rabbit	404–578		(159)
Striated muscle (red)	Rabbit	277–380		(159)
Striated muscle	Rabbit (adult)	430		(133)
Striated muscle	Rabbit (39 days)	390		(133)
Striated muscle	Rabbit (19 days)	316		(133)
Striated muscle	Rabbit (9 days)	228		(133)
Striated muscle	Rabbit (7 days)	191		(133)
Striated muscle	Rabbit (fetal)	Trace		(133)
Striated muscle	Cat	421–580	2.3– 8.0	(69) (70)
Striated muscle	Pig	364–410		(21)
Striated muscle	Ox	421–530	10.3–12.7	(21) (184)
Striated muscle (fetal)	Ox	26–290		(21)
Striated muscle	Sheep	406–419		(93)
Striated muscle	Horse	376–395		(93)
Striated muscle	Dog	408–488	5.0–15.6	(69) (184)
Striated muscle	Guinea pig	370		(133)
Striated muscle	Rat	470		(140)
Striated muscle (breast)	Hen	408–481	5.0– 5.3	(35) (140)
Striated muscle (leg)	Hen	348–368	1.6– 1.9	(35) (140)
Striated muscle	Frog	364–399		(175)
Striated muscle	Turtle	236–339		(69)
Striated muscle	Skate	280		(133)
Striated muscle	Cod	350		(133)
Striated muscle	Carp	421	77	(151)
Striated muscle	Shark	655	134	(151)
Striated muscle	*Phycis*	295–313		(35)
Striated muscle	Lamprey	290		(133)
Cardiac muscle	Ox	240–255		(21)
Cardiac muscle (fetal)	Ox	31–54		(21)
Cardiac muscle	Dog	210–327		(69)
Cardiac muscle	Cat	221–333		(69)
Cardiac muscle	Rabbit	186–291		(69)
Cardiac muscle	Sheep	208–339		(69)
Cardiac muscle	Hen	166–190		(69)
Cardiac muscle	Turtle	70–109		(69)

TABLE 1—*Concluded*

TISSUE	ANIMAL	CREATINE	CREATININE	AUTHORITY
Smooth muscle				
Uterus	Cow	35–50		(21)
Uterus (at term)	Cow	98–104		(21)
Uterus	Human	52		(21)
Uterus (at term)	Human	89		(21)
Uterus (post-partum)	Human	51		(21)
Colon	Rabbit	36		(21)
Small intestine	Rabbit	27		(21)
Gizzard	Goose	70		(156)
Bladder	Ox	99–130		(35)
Brain	Dog	108		(107)
Brain	Ox	103		(107)
Testis	Bull	195		(14)
Testis	Dog	181		(107)
Testis	Sheep	209–215		(14)
Liver	Dog	21–28	2.8	(14) (184)
Pancreas	Ox	14–23		(21)
Kidney	Ox	14–20		(21)
Spleen	Dog	15–23	4.0 (Cat)	(21) (140)
Thyroid	Calf	13	1.1	(21) (140)
Thymus	Calf	11		(21)
Blood	Man	2.1–4.9	0.7– 1.3	(103)
Blood	Hen	11	0.1	(69a)
Milk	Cow	2.0–2.6	1.0– 1.5	(53)
Milk	Human	1.9–3.9	1.0– 1.6	(54)

Table 1 includes not by any means all the data to be found in the literature upon the creatine content of tissues, but such a selection as may illustrate the chief peculiarities of its distribution. It will be seen that while noteworthy concentrations of creatine are to be found in the brain, the testes and the gravid uterus, with quantities greater still in the myocardium, no other tissue contains so high a percentage as voluntary muscle. Taking into account the average relative mass of the different organs, Bürger (32) has calculated that about 98 per cent of all the creatine in the human body is carried by the muscles, and about three fourths of the small remainder by the brain.

The creatine concentration of the muscles of a given species, among individuals chosen at random, varies within limits that are frequently

rather wide; but there are cases like that of the rabbit (140), (164) where, as the consequence perhaps of uniform conditions of feeding and environment, the muscle creatine has been found to be very nearly a constant. The variability, such as it is, does not obscure the fact that there is a certain average concentration of creatine fairly characteristic for each species.

The figures reproduced furnish evidence for some other interesting generalizations. The creatine content of muscle is not the same at all ages, but rises steadily during fetal and post natal growth till it attains the maximum characteristic of the adult. There is more creatine in the quickly contracting pale muscle than in the slowly contracting red, a difference exhibited alike by mammal and by bird. The muscle creatine of warm-blooded animals is generally speaking higher than that of the more sluggish cold-blooded, although some of the data for fish supply striking exceptions. Smooth muscle has a very much lower concentration of creatine than striped; and the gravid uterus a higher one than the non-gravid. All these facts point in one direction. They indicate very strongly that creatine is a substance with a useful function, a function in some way connected with that capacity for rapid and powerful contraction which is the most important property of striated vertebrate muscle.

CREATININE AS A CATABOLITE. Whatever the rôle of creatine may be, there is no doubt that creatinine is a mere waste product. This is demonstrated by the fact that when it is administered to animals, whether parenterally (202), (143), (176), (126) or by mouth (93), (64), (94), (216), (72), (202), (176), (172), (74), the greater part, up to 80 per cent or more, is promptly excreted. The missing 20 per cent or so has in some instances been traced to the muscles, where it has doubtless undergone the reversible transformation into creatine (143). None of it is ever converted within the body into urea or ammonia (64), (70), (172) or other known product of further degradation. Not only therefore is creatinine a waste material, but it is a terminal and not merely an intermediate product of catabolism.

The daily output of this waste product, upon a diet free from either creatine or creatinine itself, is for each individual a practically constant quantity, entirely independent of the amount of protein in the food, or of the total nitrogen catabolism, or of variations in the volume of the urine. This striking and fundamental law of metabolism, the discovery of which constitutes the real starting point of modern advance in the physiology of creatinine, was first announced in 1905 by Folin (62), and

has been fully confirmed by many later investigators (93), (43), (109), (181), (177). The "constancy" of the daily output is of course not absolute. It would appear from data scattered throughout the literature that the day to day variations may even amount to 20 or 25 per cent; but they do not often exceed 10 per cent, and are often for long periods together very much less. In any case they bear no relation to nitrogen output or diuresis.

According to Shaffer (182) the rate of elimination is uniform not only from day to day but even from hour to hour. The data of Klercker (109), Hoogenhuyze and Verploegh (95), (94), and Neuwirth (150) show no such regularity; and Schulz (177), who collected his own urine in two-hour periods for many consecutive days, found periodic variations with definite maxima, persistent even during fasting, at 9-11 a.m., 3-5 p.m. and 9-11 p.m. Schulz's data show also that the average of the night hours is lower than the average for the day. His observations were limited to one individual and it is not certain that they can be generalized. The lower rate of elimination during the night has been observed by Powis and Raper (162) and by Campbell and Webster (36), and is generally evident in the extensive data of Hoogenhuyze and Verploegh.

While the daily creatinine output is approximately a constant for the individual, it varies decidedly from one individual to another. Folin (62) recognized that the variations stood in some relation to the weight of the subject, and Shaffer (182) introduced the practice of expressing this relation by means of the "creatinine coefficient"—the number of milligrams of creatinine (or creatinine nitrogen) excreted daily per kilogram of body weight. This coefficient, expressed in terms of creatinine, varies in "strictly normal" human adults of the male sex from 18 to 32 (182); though possibly the lower limit of normality should be reduced to 15. In normal women the figures have the decidedly lower range of 9 to 26, averaging only 15.6 (203); it is probable, as Shaffer thought, that this is not an effect of sex in itself, for individual women whose muscles have been developed by gymnastic training have coefficients comparable with those of men (203). Infants and children have lower coefficients still; 6.7 to 10 at 10 to 14 days (9), and 9 to 17 at 5 to 13 years (118). The considerable range of the figures in every group shows that the relation between creatinine output and total body weight is not one of strict proportionality. What other factors intervene will be considered presently.

METABOLIC SIGNIFICANCE OF URINARY CREATININE. The constancy of the creatinine output and its utter lack of relation to the total protein metabolism were interpreted by Folin (63) as indicating that creatinine is the product of a special quantitatively unvarying form of protein metabolism, described as constituting an "essential part of the activity which distinguishes living cells from dead ones," named "*tissue* or *endogenous* metabolism," and sharply differentiated from the variable "exogenous" metabolism of the food protein. Apparently this special type of metabolism was conceived of as taking place in all the living protoplasm of the body. Differences in the creatinine coefficient were ascribed to differences in adiposity, that is, to differences in the relative amount of metabolically inert substance in the body; and the conclusion was implied, if not expressly drawn, that the creatinine output depends on the mass of active protoplasmic tissue. In his latest exposition of his views (70) Folin, indeed, states positively that he regards creatinine as an "index or measure of the total normal tissue metabolism."

In its main point, the idea of creatinine as the product of a special tissue metabolism and as having no relation to the catabolism of food protein, this conception has met with practically universal acceptance. In its details it has been variously modified. Shaffer (182) showed that there is a close parallelism between the creatinine coefficient of the individual and his muscular development, strength, or "efficiency." He suggested therefore that creatinine "is derived from, and an index of, not the total tissue or endogenous catabolism, but of one special process of this catabolism," taking place "largely if not wholly in the muscles," upon the intensity of which depends "the muscular efficiency of the individual." Similar views were expressed by Spriggs (186). There is of course an obvious probability that the production of creatinine should take place mainly in the tissue which is richest in creatine; but Shaffer's conclusions were reached independently of this consideration.

There are certain developments of Shaffer's view in which the direct derivation of creatinine from muscle creatine is taken as granted or proved. The first of these is the hypothesis of Pekelharing and Hoogenhuyze (159), which attempts to define more precisely than Shaffer did the relation between creatine or creatinine and muscular "tone." On this hypothesis the chemical transformations involved in the maintenance of tonus are considered to be entirely different from those associated with the rapid tetanic contractions of voluntary movement; while

the latter consist preëminently in the oxidation of carbohydrate, the former are thought to include changes of nitrogenous material and in particular the formation of creatine. The output of creatinine is therefore determined by the state of tone of the muscular system.

This theory was supported by a great deal of experimental evidence tending to show that muscles in a state of "tonic" contraction, brought about in a great variety of ways, gained in creatine, and that sustained contraction or "tonus" such as is involved in the maintenance of the Prussian military posture is accompanied, in contrast with ordinary muscular effort, by an increased output of creatinine (161). It may be questioned whether all the forms of continuous contraction studied by Pekelharing and Hoogenhuyze were examples of exaggerated "tonus" in the strict sense of the term. In any case Brown and Cathcart (31) obtained comparable increases of creatine in frog muscles stimulated to contract in the ordinary way, and Schulz (177) failed to confirm the alleged special effect upon creatinine output of sustained contraction in man. It is more than doubtful therefore whether the distinction drawn between the chemical mechanism of tonus and that of voluntary contractions can be taken as established. Some relation, we have seen already, probably does exist between the functional efficiency of striped muscle (one factor in the maintenance of which is tonus) and its creatine content; but as far as the evidence goes it is just as likely that tonus is dependent on creatine, as that creatine and creatinine are products of tonus.

The theory just discussed has this in common with that of Shaffer that it makes urinary creatinine an index of muscular tone. According to Myers and Fine (140) it is simply an index of muscle creatine. In a manner this is a synthesis of the two precedent hypotheses, which somewhat to its advantage leaves open the question of the significance of the creatine. Myers and Fine observed that there is an approximate proportionality between the creatine concentration in the muscles (or entire body) of an animal and its creatinine coefficient. Thus the average creatinine coefficients of the rabbit, man and dog are respectively 38.4, 24.2 and 22.5; the average percentages of creatine in the muscles of these animals are, in the same order, 0.52, 0.39 and 0.37. The coefficients stand in the ratio 1.7 : 1.07 : 1.0; the creatine concentrations in the ratio 1.4 : 1.05 : 1.0. Comparison of different individuals within a single species leads to a similar result: for 5 rabbits, with coefficients between 36 and 37, the average concentration of creatine in the entire body was 0.170 per cent; for 5 others with co-

efficients of 38 to 41 (average 40.3), the average creatine percentage was 0.193. These parallelisms strongly suggest that there is a constant relation between the total amount of creatine in the body (or, what for all practical purposes is the same, in the muscles) and the amount of creatinine in the daily urine. Direct observations upon a series of 12 rabbits showed a total body creatine ranging from 4.317 to 0.947 grams with a creatinine output of 99.9 to 19.2 mgm., while the ratio of the two lay between the comparatively narrow limits of 43.2 and 53.3 —in five cases out of the twelve between 44.4 and 45. It would appear therefore that animals with a high creatinine elimination, whatever may be their creatinine coefficient, do actually have a correspondingly high body content of creatine. All these correlations Myers and Fine interpret as indicating that urinary creatinine and muscle creatine are intimately related in metabolism, that the former takes origin either from the latter or from some common precursor, and that the relative constancy of muscle creatine in the normal animal affords an immediate explanation of the uniform rate at which creatinine is eliminated in the urine.

There are many flaws in this argument, which has been severely criticised by Benedict and Osterberg (28); but the case which Myers and Fine make out is none the less a rather strong one. The apparent demonstration of a relation, quite independent of body weight and creatinine coefficient, between urinary creatinine and total body creatine is especially to the point. The thesis has the merit of simplicity, and there are no known facts utterly incompatible with it. It has apparently been adopted by Shaffer (184) as consistent with, and complementary to, his own theory. It leaves of course unexplained the cause and meaning of the relative constancy, in individual and species, of muscle creatine.

Since all but 2 per cent or so of the body's creatine is in the muscles, it is an obvious corollary of the above theory that, so long as the muscles are in a normal state (i.e., possess a normal creatine content), urinary creatinine is determined by muscle mass, and that conversely, if once we ascertained for any species the factor connecting them, the mass of an animal's muscles could be calculated from its creatinine output. This deduction was made by Myers and Fine themselves, but, wisely perhaps, they attempted no practical application of it. Bürger (32) has recently ventured a calculation which would indicate that 1 gram of urinary creatinine per day corresponds in man to 22.9 kgm. of normal muscle. He is on surer ground when he points out that, if creatinine is

an index of muscle creatine, the creatinine coefficient becomes under physiological conditions an index of, and indeed proportional to, the participation of the muscles in the body weight. The fact that clinically, as he shows, the coefficient, in the absence of disturbing factors, does vary in a regular way with the apparent predominance of the muscles, lends a certain amount of support to the theory.

ORIGIN OF CREATININE. It was impossible to discuss the metabolic significance of the urinary creatinine without touching upon its origin; but in some respects this may be treated as an independent problem.

It was at one time believed that creatine was readily and largely transformed in the body into creatinine, and the direct derivation of the latter from the former was therefore practically taken for granted. When Folin in 1905 (64) undertook, by the aid of his newly developed colorimetric method, a study of the fate of ingested creatine, these beliefs were for the first time seriously disturbed. The essential data of three of Folin's experiments are reproduced in table 2. (In this table all reported figures for creatine have been converted into terms of the anhydrous substance as such, and a calculation has been made of the amount not accounted for in the urine as either creatine or creatinine; this is reported under the heading of "creatine retained.") The results, as illustrated, may be stated thus: *a*, creatine administration led to no increase of creatinine output; *b*, with doses of about 5 grams a certain amount of creatine was usually excreted unaltered, the proportion so eliminated being greater upon a high than a low protein diet; *c*, with small doses (1 gram or thereabout) no creatine at all reappeared; *d*, in no case was there any increase in urea or, it may be said, in ammonia, or (except once) in the undetermined nitrogen of the urine.

Such results, entirely at variance with prevailing notions, led Folin to conclude that the organism does not possess the power of converting creatine into creatinine, that these two substances are quite independent of each other in metabolism, and that creatine is not, like creatinine, a waste product, but a food.

The observation that creatine introduced from without may be partly excreted unchanged, but is usually to a large extent or even altogether retained within the organism, has been repeatedly confirmed, for animals as well as men, and not only for oral (109), (94), (216), (122), (161a), (72), (202), (118), (176), (144), (172), (74), (76), but for parenteral administration (120), (160), (202), (143), (176), (126). That none of it is ever converted into creatinine has been shown to be an error (94), (160), (202), (72), (143), (176), (144), (172), (126). If

the dose given is large enough, the increased output of creatinine is unmistakable. This is strikingly shown by two experiments of Rose and Dimmitt (172), one of which is reproduced in table 2; it will be seen that in this particular case even 1 gram of creatine produced a slight increase of creatinine, while with 20 grams the output rose to 36 per cent above the endogenous level. It may be said that in the

TABLE 2

Fate of ingested creatine

CREATINE GIVEN	TOTAL N	UREA N	CREATININE	EXTRA CREATININE	CREATINE EXCRETED	CREATINE RETAINED	AUTHOR
grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	
	5.49	4.18	1.33				
0.97	5.39	3.91	1.33	0	0	0.97	Folin
	3.99	2.80	1.34				
	3.89	2.39	1.83				
4.4 (1 dose)	3.67	1.83	1.69	0	0.84	3.56	Folin
	3.76	2.30	1.80				
	19.88	16.44	1.53				
4.4 (3 doses)	21.70	17.69	1.53	0	2.34	2.06	Folin
	20.16	16.96	1.37				
	11.05		1.35				
	10.92		1.29				
	10.42		1.35				
1	10.41		1.45	0.10	0.09	0.79	
2	10.58		1.51	0.16	0.13	1.68	
5	11.48		1.59	0.24	1.81	2.91	Rose and
10	12.83		1.61	0.26	5.81	3.88	Dimmitt
10	13.78		1.69	0.34	5.90	3.70	
20	16.86		1.83	0.48	15.10	4.34	
	11.64		1.67	0.32	0.41		
	9.87		1.61	0.26			
	11.07		1.61	0.26			
	10.40		1.52	0.17			

other experiment of Rose and Dimmitt, where urea determinations were made, there was no evidence, even after 20 grams of creatine had been given, that any of it was converted in the body into urea or ammonia. On this point Folin's conclusion is fully substantiated. Creatine therefore is not subject in metabolism to any catabolic "destruction," other than by conversion into creatinine.

The demonstration that exogenous creatine is partly transformed into creatinine may not by itself prove that endogenous creatinine has its origin in body creatine, but it disposes of the only experimental evidence irreconcilable with such an idea. The extent of the transformation is admittedly small—roughly 5 per cent of the ingested creatine; but it may be questioned if there is any real reason for expecting it to be greater. The amount of creatinine produced will depend in the first place on the active mass of the creatine at the place where dehydration occurs. There is no way of telling what that "active mass" is; but let it be assumed that it is constituted by the total creatine of the muscles, that the muscles are the sole seat of the reaction, and that accordingly only that portion of the ingested creatine which is incorporated in the muscle substance can contribute to the formation of creatinine. When creatine is introduced from without the muscles are offered an excess of a special tissue component, of which they already as a rule have a sufficient supply; small doses they may be able to absorb completely, but of a large dose much will be rejected, will on the assumption made never enter the metabolic circle, and will accordingly be excreted unchanged. Now the total creatine of the muscles amounts, for an average man, to about 112 grams (32). If that quantity be increased by the addition of 1 gram of ingested and retained creatine, it need not surprise us that the effect upon the creatinine output is imperceptible. In the second of Rose and Dimmitt's experiments (not reproduced in the table) the amount retained out of 20 grams was only 5.7, which is the largest retention ever recorded for a single day's experiment. If all of this were added to 112 grams it would mean an increase of about 5 per cent. The increase of urinary creatinine actually observed was about 0.3 gram; small as it is, it represents an excess of 18.5 per cent over the endogenous output, and is therefore more than one is forced to expect. The argument, as stated, involves several unverifiable assumptions, and in a quantitative sense deals with the crudest approximations; but in its main outline it is probably perfectly sound.

The opinion that creatinine is derived from muscle creatine is supported by the observation (184), (145), (70) that muscle contains more free creatinine than any other tissue, and more than the blood which passes through it (see table 1); as well as by the accelerated and probably enzymatic transformation of creatine into creatinine, without loss of total creatinine, which takes place in muscles or muscle extracts after death (147), (84). The first of these points has its force rather

weakened by recent evidence tending to show that blood contains no creatinine at all (20), and the second by the fact that the muscles of birds, which are said to excrete creatine in place of creatinine, show the same changes upon autolysis as those of mammals (147). It may be admitted, therefore, that no single point in the evidence, not even the partial transformation in the body of creatine into creatinine, demonstrates in a clear cut fashion the origin in normal metabolism of creatinine from creatine. Yet, when the entire body of evidence, including the apparent relations between muscle mass or muscle creatine and urinary creatinine or creatinine coefficient, is passed in review, and when it is considered that the actual ability of the body to convert ingested creatine into creatinine is no longer in doubt, it is easy to understand why the old doctrine of the origin of creatinine from creatine meets again today with practically universal acceptance.

One qualification, however, is usually present in the minds of those who subscribe to that doctrine (140), (184). The "creatine" of the living muscles may not be free creatine. There is indeed some difficulty in believing that it is free, for it is present in a concentration very much higher than that in the blood and yet the muscles are able to take up still more creatine from the circulation (70). It would seem therefore that the creatine must be held in the muscles by some special attractive force, either chemical or physical. This leads to the idea of a "creatine-containing complex," and it is possibly some such complex, rather than creatine itself, which is to be regarded as the actual precursor of creatinine. Whether in metabolism this creatine-complex, if it exists, is to be thought of as first yielding creatine, which thereupon is transformed into creatinine, or whether it yields the latter directly, is not very clear.

The second of these alternatives corresponds with the hypothesis of Folin and Denis (70), who still maintain the essential independence of creatine and creatinine; but in their view the creatine-complex is nothing short of the living protoplasm itself. The creatine of muscle, they think, is entirely a post-mortem product. The metabolism of living protoplasm gives rise directly and solely to creatinine; but at death this same protoplasm, in muscle at least, breaks down in such a way as now to liberate creatine. The chief difficulty in accepting this hypothesis is that it implies that the "death" of protoplasm is practically an instantaneous phenomenon; for no matter how suddenly an animal is killed, how rapidly or at how low a temperature its muscles are submitted to extraction, one obtains practically the maximum yield of creatine (158)

If the creatine of living muscles is in some sort of combination it must be a combination of the loosest possible kind. The case is not very different from that of the amino acids. These also accumulate in the tissues from lower concentrations in the blood (210); but it has not been suggested that they therefore become an integral part of the protoplasm, or that they are not themselves the direct precursors of urea. Although doubtless held by the tissues in some kind of loose molecular combination the amino acids behave in metabolism as if perfectly free. Probably the creatine of muscle may be regarded as doing the same.

THE INFLUENCE OF MUSCULAR WORK UPON CREATINE-CREATININE METABOLISM. The *total* output of creatinine for the day is independent not only of the quantity of protein in the diet, but also of the amount of ordinary muscular work performed (93), (181). This appears to be true even when the diet is of inadequate energy value (93). Whether it holds during a complete fast appears to be somewhat uncertain; the recent experiments of Schulz (177) throw some doubt on the conclusion drawn (from a single experiment) by Hoogenhuyze and Verploegh (93) that exercise during fasting causes a considerable rise in the daily excretion of creatinine.

It does not follow that muscular contractions have no effect at all upon creatine metabolism. An effect becomes obvious enough when the output of creatinine is measured not simply day by day, but at intervals as short as two hours. It is then found (177) that the output of any work period is decidedly and invariably greater than that of the corresponding period of a day of inactivity; and that some later period (almost always the one immediately following) exhibits with equal regularity an output unusually low. These opposing effects, which are not noticeably modified by fasting, compensate one another so closely that the net effect upon the output of 24 hours is negligible.

The temporarily increased creatinine excretion associated with work might be the consequence of an increased production of creatine within the contracting muscle, or of an accelerated transformation of the creatine already there, or of both these factors operating together. The choice between these possibilities would obviously be easier if we could learn by direct observation of the muscle itself what changes in its creatine content take place during contraction. Unfortunately no unanimity of opinion upon this point has yet been reached. It has been reported that stimulation of isolated frog muscle causes an increase of total creatinine (31); but also that it produces no effect (159), (175).

Stimulation of muscles in situ with the circulation of the blood intact has been said to bring about a decrease in the frog and in the rabbit (31); but the existence of any effect whatever under such conditions has been denied for the frog (175) and the cat (199). On the other hand cats' muscles stimulated in place after ligature of the main artery of the limb have been found to lose on the average 6.3 per cent of their creatine (199). If all the experimental data merit equal confidence, such divergence of result can have but one meaning—that in the experiments hitherto undertaken some controlling or modifying conditions have been overlooked. One cannot but be reminded in this connection of the perplexities that formerly beset the problem of the production of lactic acid in muscle; and it seems evident enough that the behaviour of muscle creatine during contraction will remain obscure, until it has been studied under conditions as precisely defined and as fully controlled as were those affecting lactic acid by Fletcher and Hopkins. It would be idle to speculate upon the facts as at present imperfectly known. One quite general deduction seems to be all that is justified; that the creatine of muscle is in *some* way, directly or indirectly, affected by muscular work. When the precise nature of the relation has been elucidated, we may be able to explain the temporary effects of work upon urinary creatinine.

THE FATE OF RETAINED CREATINE AND ITS BEARING ON CREATINE METABOLISM. We have seen that when creatine is introduced from without a certain amount is always retained in the body. The quantity which may be so retained is apparently rather limited, since even when presented with as much as 20 grams the human organism has not been observed to keep more than 5.7 grams (172). It is of interest to consider what may become of this.

Since it is certainly not destroyed, two possibilities only seem to present themselves. Either it may be deposited unchanged in the tissues, especially in the muscles, or it may be utilized in the synthesis of other substances. There is no doubt at all that the first of these alternatives can be realized. Folin and Denis (70) observed rapid increases in the muscle creatine of cats who were absorbing creatine from the intestine; in one of their experiments the creatine content of the muscle rose 26 per cent. Myers and Fine (143) were able in this way to account for from 22 to 100 per cent of the creatine retained by rabbits during continued subcutaneous administration; from which it would appear even that the *whole* of the retained creatine may sometimes be simply deposited in the muscles.

If creatinine is an index of muscle creatine, an increase of the latter brought about by the retention of ingested creatine ought to be reflected by a proportional increase in the output of creatinine, not only during the administration of the creatine, but for some time thereafter. Such a continued elimination of extra creatinine is not infrequently to be detected in the records of experiments. A striking instance is seen in the experiment of Rose and Dimmitt (172), as shown in table 2; on the fourth day after the last dose of creatine the creatinine was still decidedly above its normal level. The converse of this effect is possibly represented in the gradual fall of creatinine output which Ringer and Raiziss (166) observed to follow the complete withdrawal of creatine from the diet.

During the six days of creatine feeding in the experiment of Rose and Dimmitt the total amount of creatine retained was over 17 grams. Is it possible that this was simply added as such to the store of creatine already present in the body? As the subject weighed 54 kgm. his muscles probably contained 90 grams; 17 grams more would mean an increase of 19 per cent. The experiments of Folin and Denis, just quoted, show that as a temporary effect such an increase is possible; as a semi-permanent one it hardly seems likely. One is almost forced to assume that some of the creatine was utilized in synthetic processes. The same conclusion is indicated by the figures quoted from Myers and Fine, showing that even when the gain in muscle creatine is allowed for, as much as 78 per cent of the creatine retained may have apparently disappeared.

The evidence therefore suggests that the second alternative also is sometimes realized, that creatine may be of use in the anabolic processes of the body; may, as Folin (64) put it in 1906, serve as a food. In what manner it does so can only be guessed. Its chemical relationship to choline points to a possible use in the synthesis of lecithine; by its guanidine radicle it might be supposed to take part in the formation of arginine (158). If Cathcart (37) is right in holding that carbohydrate is necessary to its utilization, while fat is of no service, the latter supposition would be the more probable.

If creatine does function as an anabolite, its utilization is almost certainly accomplished entirely in the muscles. For in muscular atrophies or dystrophies 90 per cent or more of administered creatine, even of small doses, appears unchanged in the urine (122), (76).

Creatinuria. While the urine of the adult male on a creatine-free diet contains no creatine, that substance is a normal and constant

associate of creatinine in the urine of children of either sex up to the age of puberty. In women also creatinuria is a physiological occurrence although only an intermittent one. Both in men and in women creatinuria may be induced or increased by a great variety of experimental or pathological conditions. The lower mammals behave in these respects very much like the human species, but exhibit still more readily both the spontaneous and the induced varieties of creatinuria. Ruminants (cattle, sheep and goats), for example, regularly excrete considerable quantities of creatine (153).

The most important forms of induced creatinuria are those produced by starvation, carbohydrate deprivation (including diabetes mellitus, pancreatic diabetes, and poisoning by phlorhizin, hydrazine (127), (205), adrenalin (204) or sodium selenite (41)), wasting diseases generally, exophthalmic goiter (182), (48), fever (109), (182), (183), muscular atrophies and dystrophies (122), (76) and perhaps acidosis. As it would be impossible to consider every one of these conditions, discussion will be limited to those bearing most directly upon the physiological problem.

Creatinuria of starvation and carbohydrate deprivation. The fact that complete fasting usually brings about an excretion of creatine was first observed almost simultaneously by Cathcart (37) and F. G. Benedict (22), (23) in man, and by Dorner (55) in the rabbit. Many others have since confirmed the observation for these species (98), (26), (135), (141) or extended it to others like the dog (205), (163), (97), (99), (100), sheep (101), pig (188) and guinea pig (155). In many of the reported observations, the disturbing effect of the concomitant ketonuria upon the analysis (78), (77), (29) has been unrecognized or disregarded, but the creatinuria is nevertheless a real one (40), (138), and the only uncertainty with which the data are sometimes affected is a quantitative one.

Different individuals and different species vary in the rapidity with which fasting creatinuria sets in, and in the intensity which it assumes. In the pig it is sometimes impossible to produce a starvation creatinuria at all (130), (188). Even the dog may at certain stages of a prolonged fast excrete no creatine (100). Such differences depend no doubt upon differences in the nutritive condition of the animal at the commencement of the fast (135), or upon its relative ability to utilize fat in shielding the tissues from disintegration (130).

The creatine output during starvation bears no constant relation to either the total nitrogen or the creatinine nitrogen. Sometimes there is observed a parallelism or even a strict proportionality between

total nitrogen and "total" creatinine (135), (141), and sometimes the latter maintains an almost constant value throughout the fast, as if to suggest that fasting merely diminishes the proportion of waste creatine transformed into creatinine (22); but neither of these phenomena is a regular occurrence (218).

Starvation involves the consumption by the organism of its own protein, probably in the first place chiefly the "reserve" or "circulating" protein, but as this diminishes an increasing proportion also of the "tissue" or fully organized protein. The latter must be furnished largely by the muscles, which lose 42 per cent of their weight in a dog starved for twenty-four days. As the muscle disintegrates, its creatine, it is reasonable to suppose, will be liberated. Such internally liberated creatine might be expected to behave just like creatine introduced from without; in which case, since the starving organism is living on a fairly high protein level, some of it would be retained, but some excreted unaltered. The retained creatine would in part perhaps be utilized, in part simply deposited in the remaining muscular mass, the creatine content of which is actually found to be raised during the earlier part of a fast (136), (141). In the later stages of starvation creatine for some reason, totally unexplained as yet, is lost even from that muscle tissue which remains apparently intact (97), (141); this might explain why the creatine output rises disproportionately towards the end of the fast (100).

The direct derivation of urinary creatine from the preformed creatine of muscle was suggested by F. G. Benedict (22), was admitted by Mendel and Rose (135) as a contributing factor in hunger creatinuria, and was definitely adopted by Myers and Fine (141) as a complete explanation of that phenomenon. This view has an obvious simplicity in its favour which would make one loath to abandon it until it has been shown to be incompatible with the facts. It is supported by such an observation as that of Frontali (73) who found that the marked creatinuria which follows total thyroidectomy in dogs is accompanied by (and probably due to) a very considerable loss of creatine from the muscles. It is not invalidated by the fact that the amount of muscle disintegrated, as calculated from the output of creatine, does not often agree with the amount calculated from the total nitrogen loss. As a matter of fact muscle loss cannot be calculated from either of these data. The nitrogen of the urine is in part derived from sources, such as reserve protein or organ protein like that of liver, which yield little or no creatine, a factor which would make the proportion of creatine to total nitrogen lower in

urine than in muscle, as is usually the case. On the other hand the protein nitrogen of the disintegrated muscle may be only in part excreted, in part utilized in more essential tissues by synthetic processes which do not involve the simultaneously liberated creatine; in such circumstances the creatine output would tend to outstrip the total nitrogen, as it sometimes actually does (99). Paton (156) in 1910 not only pointed out these theoretical possibilities but attempted to illustrate them by experiments upon ducks fasted after different types of feeding and starting therefore with different amounts of surplus protein. Still other factors disturbing the relation between total nitrogen and creatine are the possible retention of muscle creatine, suggested above, and the selective loss of creatine from muscle tissue which seems to take place in the terminal stages of a fast.

Before weighing certain objections that have nevertheless been taken to Myers' and Fine's explanation of starvation creatinuria, it is necessary to consider some other aspects of the phenomenon.

In 1909 Cathcart (38) showed that the creatinuria of starvation was promptly abolished in man by the administration of a practically protein-free carbohydrate diet, but not (as he thought) by the administration of fat with protein. The effect of carbohydrate was confirmed by Mendel and Rose (135) for rabbits, and by Wolf and Osterberg (218) for dogs. Cathcart offered, to explain his results, the hypothesis that creatine is produced in the organism in considerable amounts, but is normally utilized in synthetic processes for the accomplishment of which carbohydrate is indispensable; in the absence of sufficient carbohydrate, as in starvation, the creatine, which cannot now be utilized, is excreted. Mendel and Rose, who like Cathcart found protein and fat to be ineffective, agreed that carbohydrate is specifically essential to normal creatine metabolism, but suggested that it might be concerned in the transformation of creatine into creatinine. It presently appeared, though, that the effect of carbohydrate in preventing inanition creatinuria was not so specific as had been thought. Graham and Poulton (77) showed that mere deprivation of carbohydrate, as brought about for a few days at least by a diet of fat or fat with protein, does not lead to an excretion of creatine in man; the creatine observed by Cathcart upon such diets being shown to be probably an analytical error due to ketonuria. Wolf and Osterberg (218) had already shown that in dogs serum protein will abolish the creatine of starvation almost as effectively as starch; and Rose, Dimmitt and Cheatham (171) proved that a diet of eggs (protein with fat) will do the same for man. In the

pig a pure fat diet, even if continued for a long period, does not necessarily lead to creatinuria (131), nor does a pure starch diet necessarily prevent it (188). The effect of carbohydrate therefore is neither unique nor certain. It is possible that it is, as Myers and Fine suggest (142), merely one phase of the well known sparing action of carbohydrate upon protein catabolism. The apparent impossibility of abolishing starvation creatinuria by fat alone (218) may be due partly to its relative inefficacy as a sparer of protein, partly to the acidosis which it induces, partly to the practical difficulty of feeding enough.

There are, nevertheless, certain other facts which are believed to speak for a special connection between creatine metabolism and carbohydrate. If the body tissues are deprived of carbohydrate by phlorhizin (39), (114), (115), (217), (135), (28), or lose the ability to utilize carbohydrate as in diabetes mellitus (14), (115), (191) or pancreatic diabetes (170), creatine is invariably excreted. The amounts found appear to bear an intimate relation to the extent to which carbohydrate is actually withdrawn from the tissues; in phlorhizinized starving dogs they become relatively enormous. Of course the conditions named involve not merely withdrawal of carbohydrate, but also as a rule a concomitantly increased catabolism of protein, and, as far as the data permit one to judge, the creatinuria seems under most conditions to run parallel with the latter (39), (217). S. R. Benedict and Osterberg (28) claim to have shown that it is none the less entirely independent of tissue destruction; and, as their observations constitute by far the most formidable difficulty in the way of identifying the urinary creatine of starvation or carbohydrate deficiency with preformed creatine set free from disintegrated or altered muscular tissue, they must be carefully considered.

Benedict and Osterberg found that it was possible to feed to fasting phlorhizinized dogs such amounts of creatine-free protein as would nearly or altogether abolish the negative nitrogen balance. They argue thereupon that "if the creatine of the urine has its origin in the destruction of muscular tissue . . . . . , a sparing of the body tissue destroyed by feeding exogenous protein should cause a corresponding fall in the creatine eliminated, whereas if the utilization (or destruction) of creatine be dependent upon carbohydrate utilization, the ingestion of exogenous protein in the phlorhizinized dog should not appreciably affect the output of creatine in the urine." The experimental results are held to settle quite definitely the choice between these alternatives. The creatine output, it is claimed, reveals itself as totally independent

of the state of the nitrogen balance. "Creatine may be eliminated in the urine in large amounts without any corresponding loss of body tissue." The creatine excreted cannot therefore simply represent preformed muscle creatine liberated by the dissolution of flesh. Creatine, it is concluded, must be constantly "formed in the animal organism in relatively large amounts," but "is normally for the most part either utilized or destroyed." This disposition of the creatine is dependent upon the utilization of carbohydrate, and when that is impossible, as in phlorhizinized animals, the constant production of creatine is revealed by its elimination. The success of protein in preventing creatinuria in normal dogs is attributed to its capacity for the formation of glucose; its failure in phlorhizin poisoning to the withdrawal of even proteinogenous glucose from the tissues.

It is doubtful if Benedict and Osterberg's data furnish adequate proof of these propositions. They demonstrate at the utmost nothing more than the production, independently of tissue catabolism, of notable amounts of creatine *in phlorhizinized animals*. To infer a similar extensive production in normal animals is hardly justifiable. The production in phlorhizin poisoning of excessive amounts of sugar from protein does not prove that the metabolism of amino acids in the normal organism necessarily involves the intermediate production of glucose. To show, by a drastic interference with normal metabolism, that the dog possesses the capacity to form more creatine than it can utilize, does not prove that under physiological conditions it exerts that capacity any further than the need of the organism for creatine may require. The results of Benedict and Osterberg are striking and important, and must be seriously reckoned with; but to the writer they seem to leave still open the questions of the relation between starvation creatinuria and muscle waste, and of the unique importance of carbohydrate in creatine metabolism. Their confirmation, upon pancreatectomized dogs, by Rose (170) adds of course nothing to their real significance.

It may be added that Cathcart, if the writer understands him correctly, has receded somewhat from his earlier views, and now regards the output of creatine in conditions of carbohydrate deficiency not so much as evidence of the disturbance of creatine metabolism *per se*, but rather "as an index of faulty metabolism in general" (41), (42); and he seems inclined to relate it, sometimes at least, directly to the creatine content of the muscle tissue catabolized (41).

The creatinuria of high protein feeding. In 1912 Folin and Denis (65) suggested as an explanation of the creatinuria of children that it is due to an excessively high level of protein consumption in proportion to muscular mass; but McCollum and Steenbock (130) appear to have been the first to definitely postulate an *exogenous* as well as an endogenous origin of urinary creatine, and to produce experimental evidence in its support. They came to the conclusion that not simply the plane of protein intake, but the character of the proteins in the diet, determines the extent of creatine production. They found, for example, that an abundantly fed pig receiving five times its endogenous protein requirement in the form of linseed and gluten meals excreted regularly considerable quantities of creatine, whereas if it received the same quantity of protein or even twice as much from corn alone the urine seldom contained any creatine at all. Steenbock and Gross (188) have more recently shown that in fasting pigs the administration of casein in sufficient amount will induce creatinuria when that is absent, or increase it when already present. This is in remarkable contrast to the observed inhibitory effect of serum or egg proteins upon starvation creatinuria in dogs (218) or men (171), and emphasizes the probable importance of the kind of protein fed.

Further evidence for an exogenous origin of urinary creatine has been found by Denis and her associates in the facts, *a*, that the output in a variety of conditions (exophthalmic goiter, childhood, etc.) appears to bear a relation to the meals (always creatine-free) of the subject, being very much smaller at night than during the day, and usually attaining a maximum in about two hours after the substantial meal of the day (47), (49); *b*, that the amount of creatine excreted by cases of exophthalmic goiter (male or female) is increased by high protein feeding and decreased or reduced to zero by a low protein diet (48); *c*, that the creatine output of children is increased by a diet rich in protein, and diminishes or even disappears upon a minimum protein intake (49); *d*, that creatinuria can be produced in adult women by forced protein feeding and made to disappear again by lowering the protein intake (50), (52). Gibson and Martin (76), again, found that the creatine output in pseudohypertrophic muscular dystrophy is intimately related to the protein intake, their data being of particular interest because in the condition named ingested creatine is excreted quantitatively, so that their subject was practically "diabetic" as regards creatine. They made the further very important observation that it is only the exogenous protein immediately catabolized, and not that retained for growth that affects the creatine output.

The view that creatine may, in part at least, have an exogenous origin was supported by Harding and Young (85) on the basis of data (as yet unpublished) obtained from growing puppies. On the other hand Powis and Raper (162), who first observed the periodic variations in the creatine output of children, found these quite unrelated to meals; Rose, Dimmitt and Bartlett (173) were unable to induce creatinuria in women by high protein feeding; and in the case of normal men it has not been found possible to bring about on the highest attainable plane of protein intake (even with 33 to 35 grams of nitrogen in the urine) any excretion of creatine whatever (50). Such failures do not destroy the significance of positive observations; but they emphasize the difficulty of producing creatinuria by protein feeding alone.

It is of course an apparent inconsistency that protein may sometimes, as when fed to starving dogs or men, abolish an existing creatinuria, and sometimes, as in the experiments of Denis and her collaborators, induce one where it was absent. If it is true that only that protein is effective which is catabolized, the inconsistency becomes less glaring; for much of the protein fed after a period of starvation may be simply retained in the body, as was certainly the case with the dogs of Wolf and Osterberg (218). This conception may also explain why differently constituted proteins should vary in their effect. The data for the experiments of Denis and Minot upon women (50) do not permit us to calculate exactly the nitrogen balance, but as their diets contained a large amount (50 grams) of gelatin, it is probable that the proportion of food nitrogen promptly excreted by their cases was relatively high. This may account for the difference between their results and those of Rose, Dimmitt and Bartlett.

Although an effect of food protein upon creatine production appears to have been fully demonstrated, this is not necessarily to be interpreted as proving an exogenous source for creatine, in the sense that the latter may arise directly, like urea, from certain precursors in the ingested protein molecule. There may be another explanation of the phenomenon. It may represent merely one phase of that general stimulation of cellular metabolism which is described as the specific dynamic action of protein. An increased *endogenous* production of creatine, resulting from such stimulation, might be expected to manifest itself in just the sort of creatinuria that follows protein ingestion. It would be brought about only by catabolized protein, since protein deposited in the form of new tissue exerts no specific dynamic action (125); it would be at its height, as Denis and Kramer found it to be (49), during the second and

third hours after ingestion, when heat production has reached its maximum but the output of urea is still rising (212); and it would occur most readily in those conditions where the metabolism is already relatively high, as in exophthalmic goiter, or in childhood. Such an explanation of the creatinuria following a high protein diet seems therefore to be well worthy of consideration. Protein feeding will increase also the output of uric acid; but no one has sought the origin of uric acid directly in the protein of the diet. Lewis, Dunn and Doisy (123) have shown reason to believe that proteins and amino-acids increase the production of endogenous uric acid by virtue of their general property of stimulating all cellular metabolism; it does not seem improbable that they should simultaneously increase the production of endogenous creatine.

Creatinuria in children. The fact that normal infants and children of both sexes usually excrete creatine as well as creatinine was discovered by Rose (168), confirmed by Folin and Denis (65) and by Krause (118), and has formed the subject of further study by Powis and Raper (162), Denis and Kramer (49) and Gamble and Goldschmidt (74). The creatinuria in question is of course a physiological phenomenon, and must depend upon quantitative rather than upon profound qualitative differences between child and adult. Probably it represents a temporary survival of an earlier stage in the evolution of the type of creatine metabolism which now characterizes adult man.

The precise point, or points, in the metabolic cycle, at which children differ from adult men, is not quite clear. It might be that children produce relatively more creatine; or that they produce, relatively to their muscle mass, the same quantity of creatine as adults, but have a less completely developed capacity to retain (or assimilate) it. Actually the creatine content of the immature muscle is lower than that of the fully developed (see table 1), as if it were more readily "saturated" with creatine; and in accordance with this conception children are found to excrete a relatively large proportion of ingested creatine (118), (162) —infants as much as 100 per cent (74)—just as do the subjects of muscular dystrophy (122), (76). The avidity (if it may be so expressed) of the muscle for creatine and its efficiency as a machine develop together, and as they develope creatine disappears from the urine. It is also possible that children have a relatively low power to convert muscle creatine into creatinine. On this point we have as yet no very direct evidence.

Creatinuria in women. In contrast to normal men, normal women even upon a creatine-free diet excrete, although only intermittently, small quantities of creatine. This was first observed by Krause and Cramer (116), (117), who thought that the creatinuria had a definite relation to the menstrual period. Others (167), (173), (187) have been unable to observe any regularity whatever in the output.

It appears not unreasonable to suppose that the creatinuria of women represents merely an imperfect transition from the creatine metabolism of childhood to that of vigorous adult life, and that it is associated with the relatively poor muscular development and low creatinine coefficient of the female sex in general. This would imply that the female organism has a lower power of assimilating creatine than the male. The experiments of Stearns and Lewis (187) upon the fate of ingested creatine in women seem to show that this is not the case. It is to be noted, though, that the two subjects they studied did not excrete spontaneously more than a trace of creatine, and that indeed their urine was often creatine-free for weeks together. In other words these women, in regard to creatine metabolism, gave hardly any indication that they differed from men. Perhaps, if the point were examined, it would be found, as Stearns and Lewis seem to infer, that women leading a life of abundant muscular activity exhibit no creatinuria at all.

The generally intermittent creatinuria of women becomes continuous during pregnancy (114), (117), (95), increasing toward the end so that in the last few weeks before parturition it may average 0.17 gram daily (211). This may be correlated in some way with the fact that the uterus during pregnancy acquires both absolutely and relatively more creatine that the resting organ (21). Immediately after delivery the output of creatine rises further still, sometimes reaching the extraordinary figure of 1.5 grams in 24 hours (182), and averaging 0.42 gram daily in the first four days of the puerperium (211). According to Heynemann (90), this post-partum creatinuria is at its height, in the human subject, on the third and seventh days; in the dog Murlin (139) found a maximum on the fifth day. Shaffer (182) and Murlin (139) attributed the phenomenon to the rapid escape of creatine from the involuting uterus. This explanation is extremely plausible, and one can hardly doubt that it correctly states one factor at least; but it does not seem to be certain that the gravid uterus contains enough creatine to account for the total quantity excreted. Beker (21) calculates that the human uterus in returning to the non-pregnant condition loses 0.73 gram of creatine; the total excess output of the puerperium must

often be greater than this. Mellanby (134) brings the post-partum creatinuria rather into relation with the onset of lactation. The excretion of creatine during pregnancy and the puerperium is in obvious need of further and more detailed study.

Creatinuria and acidosis. A great many of the conditions resulting in creatinuria—starvation, carbohydrate deficiency, diabetes, fever, etc.—are characterized by an abnormal production of acetoacetic acid or other acid substances, and therefore by a tendency to depletion of the alkaline reserve, or acidosis. This suggested to Underhill (206) in 1916 the hypothesis "that a condition of acidosis in the body is responsible for the appearance of creatine in the urine." After testing this hypothesis in a variety of ways upon rabbits (207), (208), (209), he concluded that there is indeed an interrelationship between acidosis and creatine elimination, but that neither acidosis nor carbohydrate deficiency can be the sole factor in the production of every type of creatinuria. A similar conclusion is reached by Steenbock and Gross (188) on the basis of experiments upon pigs. Denis and Minot (52), on the other hand, could observe no definite effect upon creatine output on administering sodium bicarbonate to boys or women. Sawyer, Stevens and Baumann (174) produced in children a demonstrated reduction of alkaline reserve by giving a high-fat low-carbohydrate diet; the creatine output was always increased, but the authors themselves do not believe that the acidosis in itself was the responsible factor. Gamble and Goldschmidt (74) were also unable to secure, by adding acid or base to the food of infants, any evidence of a relation between creatinuria and the acid-base balance of the diet.

It is quite certain that acidosis is not in any condition at present known the sole determining cause of creatinuria, and it is doubtful if it is often an important contributing one. That it may sometimes play a subsidiary part is not impossible, and may even, in view of its stimulating effect upon cellular metabolism, be considered likely.

Upon a general review of the whole situation with regard to creatinuria it appears very improbable that the condition can be referred in all of its types to any single cause. Probably creatinuria is always endogenous in origin and most examples, if not all, could be included under one or more of the following descriptions:

1. Creatinuria due to dissolution of muscular tissue; as in starvation, carbohydrate deficiency, etc.

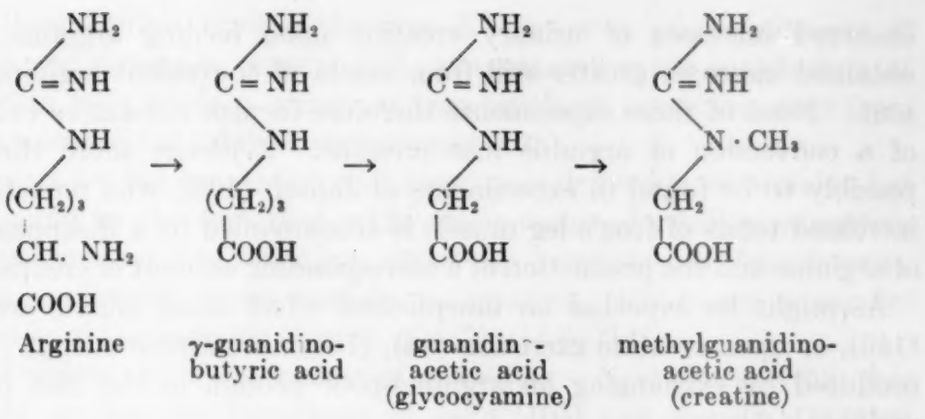
2. Creatinuria due to over-stimulation of endogenous metabolism; as in fevers, exophthalmic goiter, high-protein feeding, etc.

3. Creatinuria due to defective power to store, or utilize, or dehydrate creatine produced in normal amounts; as in childhood, muscular dystrophies, etc.

ORIGIN OF CREATINE. It is generally assumed, and the assumption has every element of probability in its favor, that creatine is derived from protein. Of the mechanism of its origin and of the nature of its immediate precursors we possess no certain knowledge. None of the supposed precursors which have hitherto formed material for experiment has been shown to satisfy the two requirements necessary to establish it in the status of an actual intermediate: *a*, that it should, upon administration in the proper way (that is, in such a way as to reach the actual locus of transformation), be converted readily and abundantly into creatine (or creatinine); and *b*, that it should be detectable in traces at least as a normal constituent of the body or of the urine. If creatine were a waste product the almost uniform failure to connect it with hypothetical precursors would almost prove that the real precursor has not yet been thought of. As we have seen, creatine is probably not a waste product, but either an integral part of the living protoplasm (70), or a tissue constituent with a special function. Its rate of production is therefore in all likelihood regulated by the internal demand, and it is not to be expected that it should be accelerated by an excessive supply of precursors, any more than the production of adrenalin or thyroxin would be increased by the administration of large doses of tryptophane. One encounters rather frequently, it is true, the idea that creatine is constantly being produced in relatively large quantities, and as constantly in some mysterious way being "destroyed." The experimental work reviewed in the preceding pages affords no proof of, and little support for, such a conception. We have positive knowledge of creatine production only in connection with the growth or renewal of protoplasm in certain tissues; and creatine is not "destroyed" in any other way than by conversion into creatinine, although it may possibly be utilized in unknown anabolic phases of metabolism. Folin and Denis (70) explain the failure to trace creatine to any known food constituent by an essentially identical argument.

Speculation and experiment upon the origin of creatine have centered mainly upon arginine, the only known protein constituent possessing a guanidine radicle.

1. Arginine as the mother substance of creatine. On the theory of Knoop (110) and Neubauer (149) creatine arises from arginine by the following series of reactions:



Up to the formation of glycocyamine this scheme follows the well established lines of amino-acid catabolism by deamination and β-oxidation. The final step in the process—the methylation of glycocyamine—is one which, it is generally agreed, the animal body has been demonstrated to be capable of taking (106), (55), (154), (197), (16), (76). The scheme has therefore a certain plausibility, increased rather than diminished by the fact that, as Neubauer himself points out, it could not be expected to apply to *ingested* arginine, the guanidine group of which would be converted into urea by the arginase of the liver, but would readily account for the appearance of creatine in the special metabolism of muscle, where arginase is not to be detected.

Attempts to secure direct evidence of the conversion of arginine into creatine have for the most part given admittedly negative or inconclusive results (106), (13), (17), (195). Positive results are reported by Inouyé (104), who obtained small increases of total creatinine upon incubation of arginine with hashed liver, or perfusion through the surviving organ; by Thompson (197) who observed increases of urinary creatine in dogs, birds and rabbits, after injection or oral administration of arginine; and by Gross and Steenbock (81) who confirmed Thompson's results by feeding the base to pigs. The significance of Inouyé's data is doubtful; but as the arginine in his experiments must have been promptly hydrolyzed by the liver arginase, its guanidine group can hardly have been responsible for any observed formation of creatine. In Thompson's experiments the extra creatine never corresponds to more than a small fraction—from 1.1 to 4.5 per cent—of the guanidine nucleus introduced; no control experiments were made with other amino-acids; and it is not at all impossible that what was observed was a general amino-acid effect of stimulation. The data of Gross and Steenbock distinctly support such an explanation; for while they

observed increases of urinary creatine upon feeding arginine, they obtained increases greater still from casein of equivalent arginine content. None of these experiments therefore furnish conclusive evidence of a conversion of arginine into creatine. Evidence more direct is possibly to be found in experiments of Jansen (108), who reports that increased tonus of frog's leg muscle is accompanied by a disappearance of arginine and the production of a corresponding amount of creatine.

As might be expected no unequivocal effect upon muscle creatine (146), or upon creatine excretion (86), (76),or creatinine output (93) is produced by exchanging an arginine-poor protein in the diet for an arginine-rich one.

In the endeavor to establish a connection between creatine and arginine, experiments have been made also with many possible intermediates and their higher homologues. Neither γ-guanido-butyric (192) nor ϵ-guanidino-caproic (193) acids, nor the corresponding methylamino or methylguanidine derivatives (194), nor yet δ-methyl-arginine (195) give any evidence of convertibility into creatine in the animal organism. Among all the conceivable intermediates of an arginine-creatine transformation glycocyamine remains the only one which gives positive results; and there are difficulties in accepting it as a real intermediate of normal metabolism. It is for the most part excreted unchanged, only a fraction of the administered dose undergoing methylation; and although only slightly soluble, it has never been detected as a constituent of animal tissues.

In the face of such almost uniformly negative results one is almost forced to assume that, if creatine is related to arginine at all, its mother substance is not free arginine but the still combined arginine of the muscle protein. Seemann's (180) hypothesis of the existence in protein of a preformed creatinine ring, in the construction of which the guanidine group of arginine is supposed to participate, contains an inherent improbability; for all the evidence indicates that the guanidine groups of the protein molecule are free (113). It is more likely that, as Thomas (195) suggests, the projecting arginine side-chains undergo in tissue metabolism β-oxidation from the terminal guanidine group inwards, the disrupted fragment being then converted into creatine. Such a speculation readily falls in line with the view of McCollum (129) that endogenous metabolism does not involve the complete disintegration of the protein molecule. Complete disintegration of the molecule would indeed be difficult to reconcile with an origin of creatine (and creatinine) from arginine alone; for arginine contributes but 11.1 per

cent, its guanidine nucleus therefore 8.3 per cent, of the nitrogen of muscle and tissues in general (56), whereas, when the metabolism of protein is reduced to a purely endogenous level, creatinine may form 18.5 per cent of the total nitrogen output (128).

Support for the conception that creatine is derived from arginine has been found in the fact that while the muscles of vertebrates contain creatine but no free arginine, those of invertebrates (crustaceans, insects, molluscs), which contain no creatine, yield larger or smaller quantities of free arginine (2), (4), (6), (7), (119), (152).

2. *Creatine as a detoxicator of guanidine.* Noel Paton (158) has recently revived the hypothesis of Jaffé (106) and Achelis (1) that creatine is the product of a reaction having for its object the detoxication of guanidine or methyl-guanidine. Guanidine is presumed to arise from the complete oxidative decomposition of arginine, from some other unknown guanidine grouping in the protein molecule, or even under certain circumstances—as in the developing chick (34)—from non-guanidine nitrogen. Methyl-guanidine is generally believed to be a normal constituent of flesh (185) and urine (57). Both bases are highly toxic, producing symptoms similar to those of tetany (157). In tetaniaparathyreopriva (112), (33) and in idiopathic tetany (33) their concentration in blood or urine or both is greatly increased. After removal of the parathyroids the creatine content of muscle increases while its total guanidine concentration falls (87). In spite of negative results (chiefly with methylguanidine) by others (106), (55), (164), (1), (17), Thompson (198) found an increase of creatinine output in the dog, and of creatine output in the duck, while Wishart (215) obtained increases of muscle creatine, after injections of guanidine salts. When it is considered that it is by no means certain (59), (18), (80) that the methylguanidine found in flesh and elsewhere is not an artificial product, the evidence hardly seems to form a very strong case for the detoxication theory.

3. *Creatine as a product of methylation.* The derivation of creatine from arginine or from the guanidine nucleus of protein involves at some point or other a process of methylation. Methylation and methylated compounds are specially characteristic of the vegetable kingdom (witness the numerous plant betaines and methylated alkaloids), but are by no means uncommon among animals. Choline (as a constituent of lecithine), adrenalin, carnitine (probably

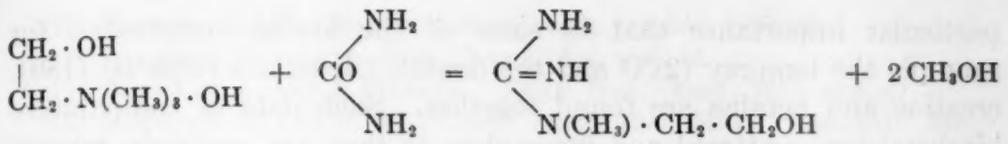
$$(CH_3)_2 : N \cdot CH_2 \cdot CH(CH_2OH) \cdot CO \cdot O$$

(58) and glycocoll-betaine are examples of methyl compounds with wide distributions in the animal kingdom. γ-Butyro-betaine is found in the urine of dogs poisoned by phosphorus (190). Pyridine (91), (96), (201), tellurium salts (92) and nicotinic acid (3) given by mouth are excreted as methyl derivatives. The instance of glycocyamine and its conversion to creatine has already been noted.

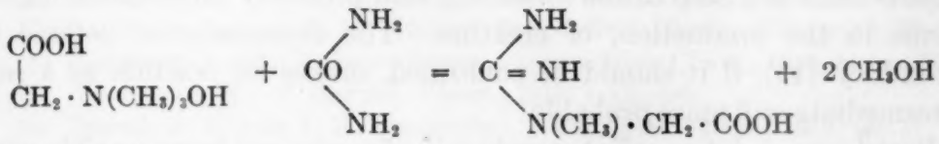
Neither the purpose nor the mechanism of methylation are perfectly clear. It has been thought that it serves to protect the substance involved from further catabolism. Certainly the betaines and methyl compounds in general are very resistant to oxidation in the animal organism (5), creatine, as we have seen, being no exception to this rule. It is possible then that the formation of creatine serves to preserve for special uses the guanidine group of arginine.

In plants methylation is believed to be effected by the union of formaldehyde with amino groups, and reduction of the methylene-amino compounds thus produced. Thompson (200) has suggested that creatine may arise in a similar way by the action of formaldehyde upon guanidino-acetic acid or some earlier stage in the oxidative catabolism of arginine. He supported his hypothesis by experiments in which the administration, especially the subcutaneous administration, of paraformaldehyde or urotropin with or without arginine to ducks appeared to give notable increases in the output of creatine. It may be objected that formaldehyde is not known to arise in the animal body, that if it did there seems to be no reason why it should couple with guanidine rather than with the far more abundant free amino groups in the tissues, and that Thompson took no account in his analyses of the fact that formaldehyde gives the Jaffé reaction. There seems therefore to be no real evidence that creatine formation represents a mechanism for the detoxication of formaldehyde, or that the latter has anything to do with its origin.

4. *Creatine as a derivative of choline or betaine.* Koch (111), in 1905, suggested that creatine as a methylated amino-acid derivative might be related to the metabolism of lecithine, of which the methylated amino-alcohol choline is a characteristic component. Riesser (164) in 1913 developed this hypothesis in considerable detail, pointing out that not choline alone but also its oxidation product betaine might be supposed to be capable of condensing with urea, according to the following schemes:



Choline



Betaine Creatine

Betaine would thus give creatine directly, while the condensation product with choline would yield it upon simple oxidation. On this theory the guanidine group of creatine would be formed synthetically, and not derived from any preformed residue in the protein molecule.

In support of his hypothesis Riesser reports experiments upon rabbits indicating that both the creatine content of muscle (164), and the daily output of creatinine (165) may be increased by subcutaneous injections of choline, or of betaine. Thompson (198), on the other hand, found no very decided effect of either substance upon total urinary creatinine in the dog; and Baumann and Hines (17), who perfused urea with betaine or choline through dog's muscle, obtained no increase of creatine with the former and only a doubtful one with the latter. The experiments of Riesser therefore, although exceedingly suggestive, do not suffice to establish either choline or betaine as precursors of creatine.

It might be pointed out that betaine at least, in the rôle of a creatine precursor, would not have any necessary connection with lipoid metabolism, but as a fully methylated glycocoll might equally well be derived from protein. Indeed it is almost certain (11) that the betaine found in living organisms arises not from choline but from glycocoll. As glycocoll can apparently be produced in almost unlimited quantities in the endogenous metabolism of protein (at the expense of a corresponding quantity of urea) (132), it is unnecessary to look elsewhere for a constant source of betaine in Riesser's hypothetical reaction. Now it is of no little interest that while betaine is absent as a rule from the creatine-containing muscles of vertebrates, it has been rather frequently found in those of invertebrates. It has been detected in the mussel (30) (7), the shrimp (2), the crayfish (119), the octopus (88), the scallop and the periwinkle (213), the spiny lobster and *Loligo* (152), none of which yield even traces of creatine. It would seem to be of

particular importance that in some of the lowest vertebrates, for example the lamprey (213) and the dogfish (*Acanthias vulgaris*) (189), creatine and betaine are found together. Such data of comparative biochemistry, scattered and incomplete as they are, certainly suggest that betaine is a step in the evolution, and probably therefore in higher forms in the production, of creatine. The occurrence of betaine in ox-kidney (19), if it should be confirmed, makes its position as a real intermediate still more probable.

Betaine as an intermediate product is of course not incompatible with arginine as the original mother substance of creatine, for arginine accompanies betaine in many invertebrates, and betaine might conceivably take origin from ornithine, through the preliminary stages of γ-amino-butyric acid and glycocoll. Methylation might be the final step, or might occur at either of the earlier stages. Carnitine, a regular constituent of vertebrate muscle, was at one time thought to be the hydroxy-derivative of methylated γ-amino-butyric acid, and as such might have figured as a possible relation of creatine; but it appears to have in reality a different constitution (58).

5. *Creatine as a derivative of cystine.* Harding and Young (86) have suggested that creatine may be derived from cystine "through the intermediate stages of taurine and amino-ethyl alcohol, followed by methylation, combination with urea, and oxidation." They have not yet published the experiments which led them to this hypothesis. Gross and Steenbock (81), who observed increases of creatine in pigs after feeding cystine, attributed their results to acidosis consequent upon the oxidation of the sulphur. Gibson and Martin (76) found cystine to be without effect upon the creatine output in pseudohypertrophic muscular dystrophy. It seems unlikely, in any case, that cystine could be the source of all the creatine produced in the body.

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